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THE RACIAL DISTRIBUTION OF NEPHRITIS AND HYPERTENSION IN PANAMA *

CARL E. TAYLOR, M.D.†

(From the Board of Health Laboratory, Ancon, Canal Zone)

In Panama a large scale natural experiment on the pathogenesis of human hypertension awaits scientific interpretation. The studies of Kean [1] and of Marvin and Smith [2] have demonstrated the presence of fairly distinct racial groups, living in contiguity and subjected to similar environmental factors, in which there is a striking difference in the incidence of hypertension. The native Panamanians originally were Indians, but in the past 300 years there has been added to this stock the blood of Spaniards and other Europeans together with their Negro slaves. This apparently composite ethnologic group is actually fairly clearly defined in language, customs, and appearance. Relatively pure-blooded Negroes were imported to Panama from the West Indian Islands for construction work on the Canal 30 to 40 years ago and with their descendants they form another rather distinct group. These racial groups were defined by Kean [1] as follows: "A 'Panamanian' is one born in Panama whose parents were both born in Panama," and "a 'West Indian' is a Negro who was either born in the West Indies of West Indian parentage or whose parents both were born in the West Indies." A third racial group is made up of Caucasians, most of whom are United States citizens.

In examining 1328 candidates for employment with the Panama Canal, Kean [1] found that hypertension was seven times as common in the West Indians as in the Panamanians; this difference was especially marked in the younger age groups in which the ratio of Negro to Panamanian hypertensive patients ranged as high as 16 to 1. In a group of almost 2,000 pregnant females he found hypertension to be five times as frequent in the West Indians as in the Panamanians. In over 2,000 consecutive hospital admissions Marvin and Smith [2] found that hypertension was about eight times as common in the West Indians as in the Panamanians.

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† Now at U. S. Marine Hospital, Pittsburgh, Pa.

Phillips [3] found a high incidence of hypertension in Negroes in Jamaica. It is the consensus of studies [4–6] of the racial incidence of hypertension in the United States that American Negroes have about twice as much hypertension as whites. Many factors have been considered in attempting to explain this difference. Heredity has been discounted because of Donnison's [7] report of the relatively low blood pressure found in African Negroes living under primitive conditions. These authors suggested that hypertension in the Negro may be caused in some way by adjustment to a new civilization and a new environment.

Shattuck [8,9] has reported that the Indians of Guatemala and of Yucatan have relative hypotension and that blood pressure in the mestizo or mixed racial groups was somewhat higher. Kean,[10] in a survey of the relatively isolated Cuna Indians living on the San Blas Islands off the coast of Panama, observed that the average blood pressure of 407 adult Indians was 105 mm. of Hg systolic and 69 diastolic; not a single case of hypertension was found.

The generally recognized correlation between hypertension and nephritis suggested that an analysis of the racial distribution of nephritis in Panama might contribute to our understanding of the problem.

Material and Methods

At the Board of Health Laboratory autopsies are performed on a large percentage of the patients dying in the hospitals of the Canal Zone and on coroner's cases and those of the police from the Canal Zone and Republic of Panama. On most of these patients fairly adequate clinical records are available. All autopsies are complete, and in all instances slides and tissue blocks have been filed and are available for examination.

In this study 800 consecutive autopsy records from 1939 to 1942 were reviewed. All cases on which there were insufficient clinical data were first eliminated, leaving a group of 498 comparable cases which in the subsequent discussion will be referred to as the *total group*. This total group was then subdivided according to race as follows: West Indian Negroes, 266; Panamanians, 77; whites, 135; miscellaneous, 20. From the total group all cases showing any sign of hypertension or nephritis were selected for special study according to the following criteria. All the blood pressure readings of each patient were averaged and if the mean level was over 140/90, or if there was any evidence of acute or chronic nephritis in gross and microscopic examinations of the kidneys, the cases were included in the *study group*.

Slides of the kidneys of the 193 cases in the study group were personally reviewed without referring to the clinical data, and the nephritic changes were classified according to the outline in Table I. This outline

is based upon pathologic descriptions in standard textbooks, except for pyelonephritis. The cases of pyelonephritis were classified according

TABLE I

Histologic Criteria for the Diagnosis of Nephritis

1. Glomerulonephritis
   - A. Acute and subacute
     - (1) Proliferative reaction in capillary tufts with hyaline material in capillary walls
     - (2) Polymorphonuclear leukocytic infiltration of capillary tufts
     - (3) Cellular and albuminous exudate in Bowman's spaces
     - (4) Epithelial crescents
     - (5) Tubular degeneration and casts (may show picture of "lipoid nephrosis")
   - B. Chronic
     - (1) Glomerular hyalinization and fibrosis
     - (2) Epithelial crescents—occasionally
     - (3) Atrophy of tubules with compensatory hypertrophy and dilatation of others
     - (4) Interstitial fibrosis and small round cell reaction
     - (5) Vascular sclerosis
2. Pyelonephritis
   - A. Acute
     - (1) Focal abscesses with polymorphonuclear leukocytic reaction
     - (2) Leukocytic casts in tubules
     - (3) Localized or diffuse acute inflammatory reaction of interstitial tissues with predominantly perivascular pattern
     - (4) Pelvic and peripelvic inflammatory reaction
     - (5) Degeneration of tubular epithelium
   - B. Active chronic
     - (1) Combination of A and C
   - C. Healed
     - (1) Pyelonephritic scars consisting of localized areas of small round cell reaction and fibrosis frequently adjacent to vessels of cortex or medulla
     - (2) Peripelvic chronic inflammatory reaction
     - (3) Periglomerular fibrosis
     - (4) Tubular atrophy with colloid casts
     - (5) Arterial and arteriolar sclerosis usually most marked in and near pyelonephritic scars
3. Nephrosclerosis
   - A. Arterial
     - (1) Medial thickening and intimal proliferation of arterial walls with or without cholesterol deposits
     - (2) Wedge-shaped scars
   - B. Arteriolar
     - (1) Subintimal hyalinization of afferent arterioles with obvious reduction of lumina
     - (2) Intimal proliferation and medial thickening of small arteries
     - (3) Occasionally necrotizing arteriolitis and proliferative endarteritis of "malignant nephrosclerosis"
     - (4) Ischemic glomerular fibrosis or hyalinization
     - (5) Tubular atrophy
     - (6) Diffuse fibrosis and slight lymphocytic reaction
4. Unclassified chronic nephritis—combination of 1, 2, and 3, defying classification

to the pathologic criteria proposed by Longcope,[11] Weiss and Parker,[12,13] Kimmelstiel and Wilson,[14] Mallory, Crane, and Edwards,[15] and Mansfield, Mallory, and Ellis.[16] In the nephrosclerotic group, sclerotic changes of only arterioles and smaller arteries were recorded. Since the gross specimens were not available it was impossible to conduct a comparative study of the larger arteries. Considerable difficulty was experienced in classifying a few cases of severely contracted kidney and also with some cases which showed slight nephrosclerotic changes but also met some of the criteria of healed pyelonephritis. All of these doubtful cases were grouped under unclassified nephritis.

No attempt was made to classify the cases on the basis of benign or malignant hypertension. In the histologic survey, however, several cases were noted that showed the necrotizing arteriolitis or proliferative endarteritis which are thought to be associated with the syndrome of malignant hypertension. These changes were observed in cases of severe pyelonephritis as well as nephrosclerosis.

Nephrosis was not included in this classification; first, because there is some doubt about the occurrence of lipoid nephrosis as a clinical and pathologic entity, and second, because the only cases of nephrosis encountered were due to acute poisoning and were not included in the study group.

Data

Racial Distribution of Nephritis

In Table II the 193 cases in the study group are classified according to race and type of nephritic change. When the original classification was made the severity of the pathologic change was graded from 1 to 4 plus. These figures were too cumbersome for presentation and the essential information was obtained by grouping grades 1 and 2 under the mild cases and grades 3 and 4 under the severe cases.

In the entire study group arteriolosclerosis is comparatively common, pyelonephritis is even more common, and glomerulonephritis is relatively rare.

West Indian Negroes. Because most of the cases of nephritis occurred in West Indians, the relative incidence of nephritis in this group does not differ significantly from that of the entire study group. Cases of nephrosclerosis and pyelonephritis are almost equally divided and together constitute 79 per cent of the total cases of nephritis in Negroes. A striking difference between these two types of nephritis is seen: 48 per cent of the cases of pyelonephritis were classified as severe, whereas only 17.6 per cent of the cases of nephrosclerosis fell into this group. It may be that the severity of the nephritis in these groups would have

been more nearly balanced if it had been possible to classify more accurately the cases of unclassified nephritis which constituted 19 per cent of the total nephritis in Negroes. Glomerulonephritis was present in only 2 per cent of the cases.

Panamanians. In spite of the fact that the total number of cases of nephritis in Panamanians is so small, it seems significant that most

TABLE II

Racial Distribution of Renal Lesions in Study Group

	Glomerulo-nephritis	Pyelonephritis				Nephro-sclerosis	Unclas-sified	Negative
		Total	Healed	Active chronic	Acute			
All races (193 cases)								
Severe	1	33	(20)	(11)	(2)	10	3	
Mild	3	39	(34)	(3)	(2)	53	33	
Total	4	72	(54)	(14)	(4)	63	36	18
Percentage	2	41	(31)	(8)	(2)	36	21	
Negroes (143 cases)								
Severe	1	25	(18)	(6)	(1)	9	2	
Mild	1	27	(24)	(3)		42	24	
Total	2	52	(42)	(9)	(1)	51	26	12
Percentage	2	40	(32)	(7)	(1)	39	19	
Panamanians (12 cases)								
Severe		2	(1)	(1)			1	
Mild	1	5	(4)		(1)	2	1	
Total	1	7	(5)	(1)	(1)	2	2	
Percentage	8	58	(42)	(8)	(8)	17	17	
Whites (34 cases)								
Severe		4	(1)	(2)	(1)	1	1	
Mild	1	7	(6)		(1)	8	6	
Total	1	11	(7)	(2)	(2)	9	7	6
Percentage	4	39	(25)	(7)	(7)	32	25	

of them are of pyelonephritis. When the 12 cases are individually analyzed it is found that 3 cases of pyelonephritis and 1 case of unclassified nephritis occurred in lepers, in whom nephritis is common, and that 2 more patients with pyelonephritis died of tuberculosis.

Whites. The most significant point to be observed is that nephritis in whites in Panama more closely resembles the general distribution of nephritis in Panama than it does the distribution of nephritis among whites in temperate zones. Only 1 mild case of acute glomerulonephritis was found in 135 autopsies. No evidence of nephritis was observed in 18 per cent of the white hypertensive patients.

Sex Distribution of Nephritis

In Table III the distribution of the cases according to sex is shown. The general autopsy rates at the Board of Health Laboratory show a preponderance of males over females of 3.3 to 1 in West Indians, 8 to 1 in whites, and 1.5 to 1 in Panamanians. When considered in relation to these rates, the proportion of nephritis in males and females is approximately what might be expected in the West Indians and whites. The high incidence of nephritis in Panamanian males is due almost entirely to the high incidence of pyelonephritis in this group. The per-

TABLE III

Distribution of Nephritis According to Sex

	Negroes		Panamanians		Whites	
	Male	Female	Male	Female	Male	Female
Glomerulonephritis	1	1		1	1	
Pyelonephritis						
Total	42	10	6	1	8	3
Healed	35	7	4	1	5	2
Active chronic	6	3	1		1	1
Acute	1		1		2	
Nephrosclerosis	35	16	1	1	7	2
Unclassified	20	6	2		7	
Negative	11	1			5	1
Total	109	34	9	3	28	6

centage incidence of cases of healed and active chronic pyelonephritis in the nephritic groups that were large enough to permit analysis was: black males, 37.6 per cent; black females, 29.4 per cent; and white males, 21.4 per cent. The incidence of nephrosclerosis in the same groups was: black males, 32 per cent; black females, 47 per cent; and white males, 30.4 per cent.

Age Distribution

The average ages of the various races in the study group were: Negroes, 56.8 years; Panamanians, 47.3 years; and whites, 55.2 years. Although the Panamanians are apparently younger than the other racial groups, there are so few cases that the standard error of their average age is $\pm 2 \times 7.87$ which brings this difference well within the range of a chance occurrence.

Incidence of Nephritis in Total Group

Table IV and Text-Figure 1 contain the most significant data presented in this paper. The percentage incidence of the various types of nephritis in the total group and the average blood pressures for each group are shown. The incidence of each type of nephritis within a

racial group is shown best in Table II, while in Table IV the incidence of a specific type of nephritis in one race can be more readily compared with its incidence in the other racial groups.

Among the West Indians autopsied, 49.3 per cent had some form of nephritis in contrast to 15.6 per cent of the Panamanians and 20.7 per

TABLE IV

Nephritis and Blood Pressure in Total Autopsy Group

	Negroes (266 cases)			Panamanians (77 cases)			Whites (135 cases)		
	Cases	Per-centage	Blood pressure	Cases	Per-centage	Blood pressure	Cases	Per-centage	Blood pressure
Glomerulo-nephritis	2	0.7	150/85	1	1.3		1	0.7	140/90
Pyelonephritis	52	19.6		7	9.1	—	11	8.2	
Healed	(42)	(15.8)	178/107	(5)	(6.5)	136/86	(7)	(5.2)	160/92
Active chronic	(9)	(3.4)	165/94	(1)	(1.3)	151/84	(2)	(1.5)	149/83
Acute	(1)	(0.4)	165/80	(1)	(1.3)	117/70	(2)	(1.5)	150/85
Nephrosclerosis	51	19.2	180/107	2	2.6	145/100	9	6.7	168/108
Unclassified	26	9.8	172/101	2	2.6	133/96	7	5.2	155/98
Total nephritis	131	49.3	176/104	12	15.6	136/89	28	20.8	159/98
Negative	12	4.5	156/95	0			6	4.4	155/96
Total	143	53.8	174/103	12	15.6	136/89	34	25.2	158/97

Standard Errors of Differences in Percentages

Total Nephritis:
Negro to Panamanian $\pm 2 \times 5.15$
Negro to White $\pm 2 \times 4.64$
White to Panamanian $\pm 2 \times 5.42$

Pyelonephritis:
Negro to Panamanian $\pm 2 \times 4.02$
Negro to White $\pm 2 \times 3.37$

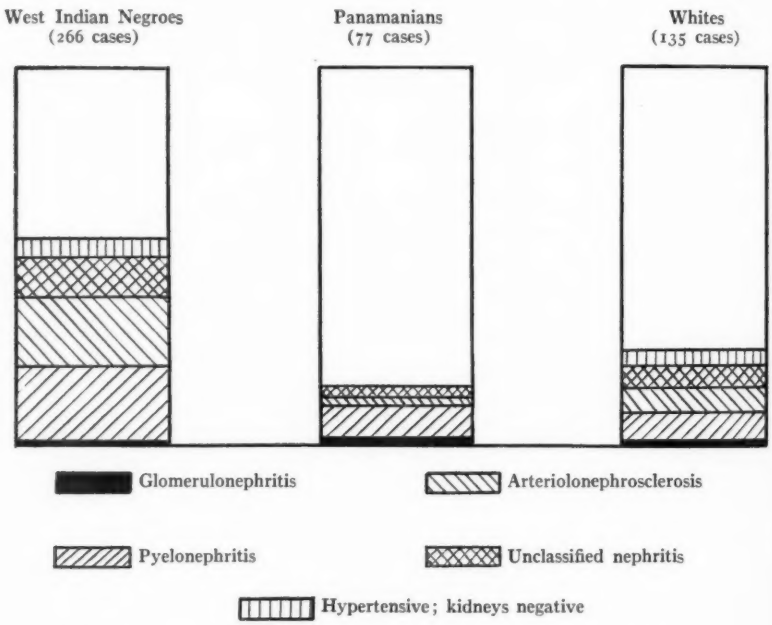
Nephrosclerosis:
Negro to Panamanian $\pm 2 \times 3.02$
Negro to White $\pm 2 \times 3.24$

cent of the whites. This marked difference is evident in all of the specific types of nephritis except glomerulonephritis, which is equally low in all groups. The standard errors of the differences in the proportions show that all differences between the Negroes and the Panamanians, and between the Negroes and whites are valid, while the differences between the Panamanians and whites may be due to chance. Pyelonephritis is twice as common in the West Indians as it is in the other two races; this difference is especially noticeable in healed pyelonephritis which occurred three times as often in the West Indians as in the other groups. Arteriolonephrosclerosis is more than seven times as common in the Negroes as in the Panamanians and three

times as common in the Negroes as among the whites. The only other point to be noted is that almost 5 per cent of the total number of West Indians and whites had hypertension of slight degree without renal pathologic changes.

Correlation of Nephritis with Blood Pressure

Table IV shows that the highest blood pressures were recorded in Negroes with healed pyelonephritis and in whites and Negroes with nephrosclerosis. It is fairly consistently true in all groups that the West



Text-Fig. 1. Percentage incidence of nephritis in total autopsy group.

Indians had the highest blood pressures, the Panamanians had the lowest, and the whites fell between. The average blood pressures of the nephritic patients were: West Indians, 176/104; Panamanians, 136/89; and whites, 159/98. A correlation between the severity of nephritis and the blood pressure is evident in the following groups, which are the only ones large enough to analyze: Negro, pyelonephritis, severe, 187/110; and mild, 171/104; Negro, nephrosclerosis, severe, 189/110; and mild, 177/105.

The relative incidence of hypertension in the racial groups also may

be demonstrated by computing the percentage of patients in the total groups with diastolic blood pressures over 100 mm. of Hg, as shown in Table V. According to this criterion there were six times as many hypertensive patients among the Negroes as among the Panamanians, and three times as many among the Negroes as among the whites.

Causes of Death in Study Group

Table VI shows the causes of death in the hypertensive and nephritic cases classified according to the International List of the Causes of Death. It is apparent that most of the West Indians and whites died of conditions which were related to the cardiovascular or renal systems. Most of the Panamanians died of acute or chronic infections.

TABLE V

Incidence of Hypertension

(Cases with Diastolic Blood Pressure over 100 mm. of Mercury)

	Negroes	Panamanians	Whites
Number of cases	87	4	14
Percentage of total group	32.7	5.2	10.4
Percentage of nephritic group	66	33	50

REVIEW OF THE LITERATURE ON RACIAL INCIDENCE OF NEPHRITIS

General

The recognition of the co-existence of nephritis and hypertension is almost as old as the acceptance of these conditions as distinct clinical entities, but only recently has there been significant progress in understanding the causes for this relationship. The concept of renal hypertension which has grown out of much recent experimental and clinical evidence has stimulated many attempts to clarify the much argued pathologic classification of nephritis, particularly since the introduction of pyelonephritis as a cause of hypertension.

Lewis [5] has stated: "The literature on kidney diseases discriminates very little between those occurring in Negroes and in whites. The chief source of information is the various mortality statistics . . . Most of the mortality statistics fail to distinguish between the various forms of nephritis." In 1930 the mortality statistics for all the states showed one-sixth as much acute nephritis, about three-fourths as much chronic nephritis, and one-third as much unspecified nephritis in whites as in Negroes. Lewis noted that "it is clear that in each state the mortality of Negroes is much higher than that of whites." Statistics of the

Metropolitan Life Insurance Company,[17] 1911–1935, also showed a third as much acute nephritis and half as much chronic nephritis in whites as in Negroes, the rates for males and females being approximately equal.

In African Negroes, Hennessey [18] found that both cardiovascular diseases and nephritis were relatively uncommon, the most common

TABLE VI
Causes of Death in Study Group

	Negroes	Panamanians	Whites	Other races	Total
Hypertension and cardiac failure	26	1	4	1	32
Cerebral accident	19		5	2	26
Coronary accident	11		2		13
Pyelonephritis	12		1	1	14
Nephrosclerosis	1				1
Glomerulonephritis	1				1
Arteriosclerotic gangrene	3				3
Aneurysm	4				4
Malignant tumor	19		5		24
Cirrhosis	1		3		4
Pneumonia	7	1	1		9
Tuberculosis	6	2	1		9
Malaria		1	2		3
Leprosy	3	4			7
Syphilis	5	1			6
Typhoid and enteritis	2		1		3
Diabetes	2		1		3
Meningitis	2	1			3
Accidental	5		3		8
Blood dyscrasia			1		1
Thyrotoxicosis	1				1
Liver abscess	1				1
Surgical conditions of abdomen	12	1	4		17
Total	143	12	34	4	193

fatal nephritis being "proliferative hyalizing glomerulitis." Donnison [7] found no nephritis in 1800 native African hospital patients. In Yucatan and Guatemala, Shattuck [8,9] found a low incidence of nephritis among the native Indians. In Panama the only previous approach to this question was Marvin and Smith's [2] observation that of their hypertensive patients 94.1 per cent of the Panamanians and 83.3 per cent of the West Indians had abnormal urinary constituents. Of their patients, 84.4 per cent also showed diminished excretion of phenolsulfonephthalein.

Glomerulonephritis

In spite of the intensive study which glomerulonephritis has received, there is very little information available on its geographic and racial distribution. Seegal, Seegal, and Jost [19] found that in four latitude areas in the United States there was no significant variation in the incidence of

acute glomerulonephritis. This was in sharp contrast to the decreased case rate of scarlet fever and rheumatic fever in the southern states.

Pyelonephritis

Longcope [11,20] and Weiss and Parker [12,13] have demonstrated that hypertension may develop in cases of active chronic and healed pyelonephritis. Their case studies are so convincing that there is little doubt that a causal relationship exists. It is, however, difficult to find exact data on the percentage of cases of hypertension which are initiated by pyelonephritis and there are very few studies which have taken racial differences into consideration. Weiss and Parker [12] felt that 15 to 20 per cent of cases of malignant hypertension were due to pyelonephritis. Dunn [21] found that patients dying of hypertensive heart disease had pyelonephritis in the following percentages: white men, 13.8; Negro men, 22; white women, 9.8; and Negro women, 42.6. Kinney and Mallory [22] found healed pyelonephritis in 14 per cent of 1,000 consecutive post-mortem examinations at the Boston City Hospital.

Most studies [23-27] show that a larger percentage of patients with pyelonephritis have hypertension than is found in control groups or in groups with other types of renal disease. In some studies,[28,29] however, no correlation between hypertension and pyelonephritis was found. Many reports [24,25,30,31] of an improvement in the hypertensive syndrome following the ablation of a diseased kidney have now appeared. Even such sceptics as Smith, Goldring, and Chasis [32] accepted some of these case reports although they threw out most of them as being inconclusive.

Various estimates of the importance of chronic pyelonephritis as a cause of the contracted kidney of chronic Bright's disease have been made. Weiss and Parker [12] stated that 30 to 35 per cent of bilateral contracted kidneys were due to pyelonephritis. Ellis [33] found 13 examples of chronic pyelonephritis in 45 contracted kidneys. Staemmler [34] classified 55 contracted kidneys as follows: nephrosclerosis, 27; pyelonephritis, 18; and glomerulonephritis, 10.

Nephrosclerosis

Moritz and Oldt [35] found that 97 per cent of hypertensive patients had renal arteriolosclerosis and the remaining 3 per cent had severe renal arteriosclerosis. In contrast to this, only 12 per cent of nonhypertensive patients had arteriolosclerosis and the vascular lesions in all but 2 per cent of these cases were mild. A point of particular interest in relation to this study was their finding of 50 per cent more deaths due

to hypertension in Negroes than in whites. Other pathologic studies [27] have corroborated this correlation between renal arteriolosclerosis and hypertension. In a study of severely hypertensive patients whose kidneys were examined by biopsy in connection with operations for sympathectomy, Castleman and Smithwick [36] concluded that renal arteriolosclerosis was probably secondary to hypertension. Seven per cent of their patients showed no nephrosclerosis and in 53 per cent the vascular changes were so slight that they scarcely could have caused sufficient renal ischemia to produce hypertension. They did not study forms of nephritis other than nephrosclerosis.

Discussion

In a comparison between these published data and the findings in Panama several significant facts become evident. Nephritis in Panama differs from that in temperate zones, first, in the relative absence of glomerulonephritis. Almost all of the cases of glomerulonephritis in this series were mild and acute, and in only one case was it recorded as the cause of death. No cases had progressed to show sufficient evidence of chronic glomerulonephritis to permit that diagnosis histologically. This is probably associated with the fact that most manifestations of streptococcal infections are generally believed to be both rare and mild here. Tucker, Miller, and Kean [37] have found that the incidence of scarlet fever in Panama is lower than in the United States and that the diagnosis is made five times more frequently in whites than in Negroes.

Pyelonephritis is the most common form of nephritis in these patients. As would be expected in autopsy reports, most of the cases are of healed or active chronic pyelonephritis. This high incidence of pyelonephritis is most marked among the few Panamanians who had nephritis and almost all of these patients also had a concurrent chronic infection such as leprosy or tuberculosis. Thirty-two per cent of the nephritis in whites is due to healed or chronic pyelonephritis, which is about the same as the published estimates of the number of cases of chronic Bright's disease which are caused by pyelonephritis elsewhere. The incidence of pyelonephritis is even higher, 39 per cent, among the West Indians. This difference between whites and Negroes is not as marked as in Dunn's [21] report, and the distribution according to sex is the converse of that shown in her data. The racial difference is accentuated, however, when the percentages are derived using the total group of patients as a base rather than the study group. The percentage of Negroes with healed and active chronic pyelonephritis is

19.2 as compared with 6.7 per cent of the whites. The reason for this striking racial difference is not apparent. It is probably not a matter of difference in hygiene, cleanliness, sanitation, or prevalence of venereal disease because the incidence rate is approximately the same in Panamanians and whites, and if there is any difference between Negroes and Panamanians in any of these factors this difference probably favors the Negroes. The average blood pressures of the racial groups with pyelonephritis also show significant differences. The blood pressure of the Negroes is somewhat higher than that of the whites and considerably higher than that of the Panamanians. This suggests that other factors may be responsible for the development of hypertension in the Negroes and whites, or possibly that there is a varying intrinsic susceptibility in the races. However, this may be merely a reflection of the severity of the pathologic change. Among the Negroes, 48 per cent of the cases of pyelonephritis were classed as severe as compared with 36 per cent in the whites and 28 per cent in the Panamanians.

The most marked racial difference in the incidence of any form of nephritis is in the occurrence of nephrosclerosis in Negroes and Panamanians. The fact that this condition is over seven times as common in the Negroes as in the Panamanians is a significant corollary to the similar ratio in the incidence of hypertension in these groups. The whites again occupy an intermediate position. The differences in the blood pressure levels of the racial groups are about the same as with pyelonephritis. Again a similar difference in the severity of nephritis is observed.

Another point that merits consideration is the occurrence of hypertension in the absence of nephritis. Among both the Negroes and whites this is true in almost 5 per cent of the total number of cases autopsied or in 12 per cent and 18 per cent respectively of hypertensive patients. The hypertension in these cases was mild. This suggests that "essential hypertension" may occur in these two groups, while if a Panamanian develops hypertension it is probably on a renal basis.

Conclusions

Previous studies have shown that hypertension is five to eight times as frequent in West Indian Negroes living in Panama as in native Panamanians. From this analysis of 498 comparable autopsy cases the following conclusions seem to be justified.

1. Among the Negroes, hypertension (diastolic blood pressure over 100 mm. of Hg) was six times as common as in Panamanians and three times as common as in whites.

2. Among the Negroes there was three times as much nephritis as among the Panamanians and almost two and one-half times as much as among the whites.

3. The average blood pressures of the nephritic patients were: West Indians, 176 mm. of Hg systolic and 104 diastolic; Panamanians, 136 mm. systolic and 89 diastolic; and whites, 159 mm. systolic and 98 diastolic. Although this may mean that there is an actual difference in the susceptibility of the races to hypertension, it is also true that the severity of the nephritis in the three races showed a general correlation with the level of the blood pressure.

4. Glomerulonephritis comprised about 1 per cent of the nephritis in all racial groups and was never recognized in its chronic stages in this series.

5. Pyelonephritis was the most frequent form of nephritis in all groups and it was twice as common among the Negroes as among either of the other groups.

6. Healed pyelonephritis was three times as common among Negroes as among either of the other two groups.

7. Pyelonephritis comprised 58 per cent of the nephritis in Panamanians and most of these patients had concurrent leprosy or tuberculosis.

8. There was more than seven times as much arteriolonephrosclerosis among West Indians as among Panamanians and three times as much as among the whites.

9. No significant correlation between nephritis and sex distribution was observed.

10. Five per cent of the Negroes and whites had mild hypertension with no evidence of nephritis.

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EXPERIMENTAL STUDIES IN CALCIFICATION

IV. The Effect of Irradiated Ergosterol and of Starvation on the Dentin of the Rachitic Rat *

J. P. Weinmann, M.D., and I. Schour, D.D.S.

(From the Department of Research of School of Dentistry of Loyola University, Chicago College of Dental Surgery, and the Department of Histology, University of Illinois College of Dentistry, Chicago, Ill.)

McCollum, Simmonds, Shipley, and Park,[1] in 1922, discovered that starvation had the same healing effect on the rachitic epiphyseal cartilage as the administration of suitable amounts of cod-liver oil. It seemed, therefore, of interest to investigate the reaction of rachitic dentin to starvation and to compare the effect with that produced by vitamin D.

Review of the Literature

Weinmann,[2] in 1930, reported the changes in the dentin of rachitic rats following daily administration of 5 mg. of vigantol (vitamin D). The first effect was seen 1 day after administration in the incisal portion of the incisor where the pulpal layer of the dentin was deeply stained with hematoxylin. Under continued administration of vitamin D the hypercalcified layer extended toward the middle portion of the incisor. The basal predentin which was very long in rickets showed progressive calcification following the administration of vitamin D.

Irving[3] studied the dentin of rachitic rats 10 days after administration of 9.2 international units of vitamin D. He found that the dentin matrix laid down 4 to 6 days after administration was deeply stained with hematoxylin and that the predentin laid down prior to vitamin D treatment was either completely unchanged or else showed a few scattered calcified globules.

No references are available on the effect of starvation on either the normal dentition or that in rachitis.

Material and Methods

This report is based on the study of the upper jaws of four groups of white rats † which were sacrificed at 49 to 52 days of age. Group I: Four rats were fed a rachitogenic diet for 4 weeks (Steenbock and Black[4]) and were given 3 cc. of irradiated ergosterol 1 to 4 days before sacrifice (Table I). Group II: Four litter-mates of the animals of group I were fed a rachitogenic diet for 4 weeks and were starved for a period of 1 to 4 days (Table II). Group III: Three rats fed a

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† The animals used in this series of study were kindly made available by Drs. F. C. McLean and W. Bloom.

rachitogenic diet after weaning. Group IV: Three rats fed a basal diet. The control groups III and IV included litter-mates of groups I and II.

The heads of the experimental and control rats were removed and fixed in 10 per cent formalin immediately after sacrifice. The jaws were dissected and the upper incisors and molars were prepared for de-

TABLE I

Data on Four White Rats Placed on a Rachitogenic Diet and Then Given 3 cc. of Irradiated Ergosterol

Number	Age when sacrificed	Duration of rachitogenic diet	Time elapsed after last administration of ergosterol
	(*days*)	(*days*)	(*hours*)
2904	49	28	10
2905	50	29	22
2906	51	30	46
2907	52	31	70

calcified histologic sections. The incisors were cut in serial longitudinal sections and the molars in serial mesiodistal sections. The stains used were hematoxylin and eosin.

FINDINGS

Fasting or the administration of vitamin D resulted in an improvement in the calcification of the dentin. This effect was best observed in the basal predentin of the incisor. In this tooth a layer of predentin

TABLE II

Data on Four White Rats Kept on a Rachitogenic Diet and Subjected to Fasting

Number	Age when sacrificed	Duration of rachitogenic diet	Duration of fasting
	(*days*)	(*days*)	(*hours*)
2908	49	28	12½
2909	50	28	36½
2910	51	28	60
2911	52	28	84

is normally found along the entire pulpal surface as long as the latter is lined by active odontoblasts. The predentin layer is adjacent to the calcified dentin except at the most basal end where the predentin is next to the dentino-enamel or dentino-cemental junction. In this region the predentin just recently deposited has not yet calcified. This basal portion of the predentin layer is normally 0.1 to 0.2 mm. in length and will be referred to as the basal predentin (Fig. 1).

In rachitic animals the basal predentin is of considerable length, especially on the cementum-covered side of the tooth [5] (Fig. 2).

The earliest changes following the administration of vitamin D were seen at the end of the first day and, after fasting, on the second day. These consisted of an improvement in the calcification of the predentin, especially the basal predentin (vitamin D, Fig. 3; and starvation, Fig. 5). One or 2 days later the calcification of the outer layer of the basal predentin extended almost to the basal end of the incisor (Figs. 4 and 6).

Intermediate Dentin. A small zone of intermediate dentin is present in the middle third of the enamel-covered wall in the incisor of the normal rat but is missing in almost all rachitic rats. This layer of intermediate dentin reappeared beginning with the end of the first day of fasting and on the second day of vitamin therapy. It extended also to the incisal half of the basal third (Figs. 9 and 11). The predentin next to the intermediate dentin contained isolated globules (Figs. 9 and 10) which stained like the intermediate dentin (globular predentin, Schour and Rogoff [6]).

Incisal Dentin. In the incisal third of the incisor the dentin immediately adjacent to the predentin stained deeply with hematoxylin. This stripe increased in width and might extend into the middle third of the tooth with increase in the duration of the experiment.

Discussion

That fasting causes a reaction in the rachitic dentin identical to that induced by vitamin D is in complete agreement with the findings of McCollum *et al.*[1] on the changes in the epiphyseal cartilage. In both cases vitamin D or fasting improved the calcification of affected tissues. We can fully agree with their explanation of the effect of fasting on healing of rickets. They stated: "Just as soon as the load of a defective diet is removed and the body is forced to draw on its own tissues for maintenance of life and function, stored foodstuffs are released into the blood stream as the result of a process of selective tissue decomposition."

Cavins [7] noted that the fasting of rachitic rats induces a sharp rise in the level of inorganic phosphorus of the blood. In the light of our findings [8] and those of McLean and McCoy,[9] that following injections of phosphate solution the uncalcified hard tissues of rachitic rats started to calcify, it can be assumed that the liberation of phosphate compound from the tissues induces calcification in fasting rats.

The basal predentin is an excellent site for the study of the beginning of calcification of dentin. The earliest attempt at calcification starts in the oldest layers of the rachitic predentin.

The fact that the layer of intermediate dentin disappears in rickets

and reappears in our experiments which initiate a healing of the rachitic condition suggests that intermediate dentin can be considered a stage in the normal calcification of dentin. Its normal presence in the middle third of the convex wall of the incisor seems to show that in this region the conditions for the calcification of the predentin are normally more favorable than in the other parts of the tooth. In this zone the peripheral layer of the predentin has undergone partial calcification, while the predentin in other parts of the tooth is uncalcified in its entire width. The calcification in this area is more rapid and the predentin, therefore, is narrower. In fact, the width of predentin plus intermediate dentin is equal to the width of the predentin in the basal and incisal areas.

The preferred location of the intermediate dentin in the middle third of the tooth in normal rats may be explained on the basis of the vascular supply of this region. It is known that calcification is retarded in richly vascularized areas and in areas lying in close proximity to blood vessels. The vascularization of the middle third of the pulp seems to be optimal for calcification because it is not as rich as that of the basal third, whereas the blood vessels in the incisal third of the pulp show regressive changes.

The fact that the calcification of rachitic dentin improves during fasting may explain in part the inconsistency in calcification observed in the dentin of various experimental animals. The possible effects of spontaneous fasting of animals during short-term experiments must be kept in mind.

Our experiment was restricted to a period of 4 days following the first administration of vitamin D. The observation that an attempt at calcification in this period starts in the oldest layers of rachitic predentin does not contradict the findings of Irving [3] who investigated the incisor 10 days after administration of vitamin D. He wondered why "the old predentin was not more altered, seeing that this was being calcified, though with difficulty, while the animal was on the deficient diet alone." However, his photomicrographs of fields taken from the middle and labial portion of the incisor show a layer of intermediate dentin adjacent to the peripheral layer, which calcified during the rachitic regime.

Summary

The dentin of incisors of four albino rats placed on a rachitogenic diet and given 3 cc. of irradiated ergosterol, and of four litter-mates kept on a rachitogenic diet and subjected to fasting for different periods was studied histologically. The histologic findings were:

1. Fasting and the administration of vitamin D cause identical reparative changes in the dentin of rachitic rats.

2. The first calcification of the wide rachitic predentin begins in its oldest layer and is best seen in the basal predentin.

3. The layer of intermediate dentin which has disappeared during rickets redevelops in the basal third of the enamel-covered dentin.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 184

Photomicrographs of longitudinal sections of the basal end of the lingual side of upper incisors. The area is indicated in the insert in Figure 1. B.pd. = basal predentin; Od. = odontoblasts; Pdm. or Per. = periodontal membrane; Al.B. = alveolar bone; P = pulp. × 48.

FIG. 1. Rat 2901, 49 days of age, placed on normal basal diet. The predentin is thin and the basal predentin is of very short extent (0.1 mm.).

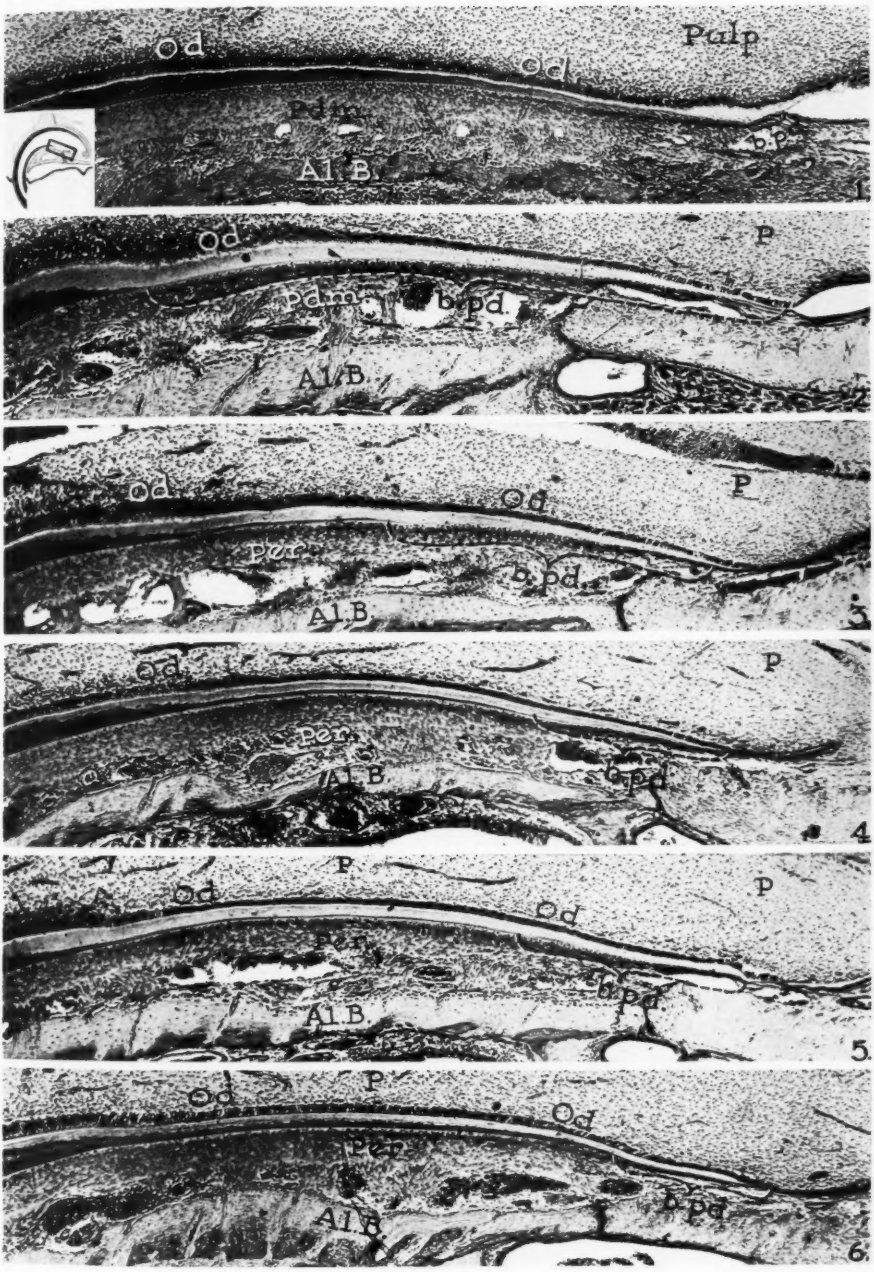
FIG. 2. Rat 2903, 49 days of age, placed on rachitogenic diet for 28 days. Here are seen the thick layer of predentin; the long basal predentin (1.5 mm.); and the narrowing of the width of the dentinal wall at the area where calcification of the dentin starts.

FIG. 3. Rat 2905, 50 days of age, placed on rachitogenic diet for 29 days, and given 3 cc. of irradiated ergosterol 22 hours before sacrifice. A thin stripe of calcified dentin extends halfway into the basal portion, which is otherwise formed by basal predentin only (0.8 mm.).

FIG. 4. Rat 2906, 51 days of age, placed on rachitogenic diet for 30 days and administered 3 cc. of irradiated ergosterol 46 hours before sacrifice. The increased length of the calcified stripe in the otherwise uncalcified basal portion (0.6 mm.) may be compared with that in Figure 3.

FIG. 5. Rat 2909, 50 days of age, placed on rachitogenic diet for 28 days and subjected to fasting for 36½ hours. There is narrowing of the dentinal wall and a thin stripe of calcified dentin in the basal portion. The basal predentin is 0.7 mm. long.

FIG. 6. Rat 2911, 52 days of age, placed on rachitogenic diet for 28 days and subjected to fasting for 84 hours before sacrifice. The increased calcification in the basal portion may be compared with that in Figure 5. The basal predentin is 0.4 mm. long.



Weinmann and Schour Effects of Ergosterol and Starvation

PLATE 185

Photomicrographs of longitudinal sections from the basal third of upper incisors. The area is indicated in the insert in Figure 7. D = dentin; Pd. = predentin; Od. = odontoblasts; I.D. = intermediate dentin. × 610.

FIG. 7. Rat 2901, 49 days of age, kept on basic diet. Of note are the wide, fairly evenly calcified portion of the dentin and the narrow stripe of predentin.

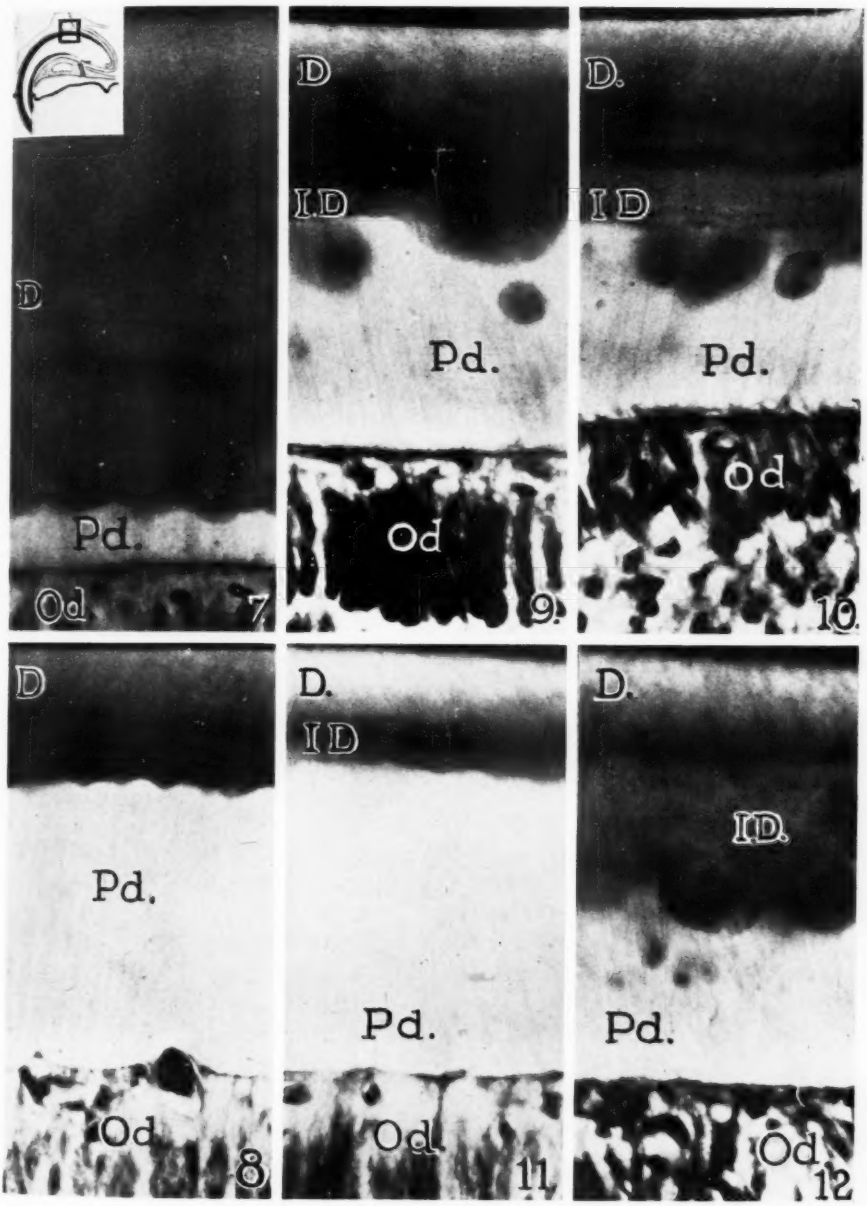
FIG. 8. Rat 2903, 49 days of age, placed on rachitogenic diet for 28 days. The extensive width of the predentin, the narrow portion of the calcified dentin, and the reduced width of the total dentin wall may be compared with the normal appearance of Figure 7.

FIG. 9. Rat 2905, 50 days of age, placed on rachitogenic diet for 29 days and given 3 cc. of irradiated ergosterol 22 hours before sacrifice. A layer of intermediate dentin adjacent to the peripheral calcified dentin and globuli in the predentin are shown.

FIG. 10. Rat 2907, 52 days of age, placed on rachitogenic diet for 31 days and given 3 cc. of irradiated ergosterol 70 hours before sacrifice. The layer of intermediate dentin shows an increase in thickness and globuli are present in the predentin.

FIG. 11. Rat 2909, 50 days of age, placed on rachitogenic diet for 28 days and subjected to fasting 36½ hours before sacrifice. There is a layer of intermediate dentin adjacent to the pre-experimental calcified layer of dentin.

FIG. 12. Rat 2911, 52 days of age, placed on rachitogenic diet for 28 days and subjected to fasting 84 hours before sacrifice. To be noted are the increase in thickness of the layer of intermediate dentin and the reduced thickness of the predentin.



Weinmann and Schour Effects of Ergosterol and Starvation

EXPERIMENTAL STUDIES IN CALCIFICATION

V. The Effect of Phosphate on the Alveolar Bone and the Dental Tissues of the Rachitic Rat *

J. P. Weinmann, M.D., and I Schour, D.D.S.

(From the Department of Research of School of Dentistry of Loyola University, Chicago College of Dental Surgery, and the Department of Histology, University of Illinois College of Dentistry, Chicago, Ill.)

Elsewhere [1,2] we have described in detail the rachitic changes in the dentin of the rat incisor and have made an attempt to interpret the development of the rachitic deformities of the alveolar bone by studying the pathologic changes in those areas in which the normal growth pattern had been carefully analyzed. The purpose of this study was to trace the beginning of healing in selected areas of the rachitic alveolar bone and in the rachitic dentin following injections of phosphate solutions.

Review of Literature

Lilly, Peirce, and Grant [3] reviewed the literature, up to 1935, on the effect of phosphorus on bones. They observed that healing of rickets by feeding phosphates is comparable to that produced by vitamin D.

McLean and McCoy [4] injected sublethal doses of phosphate (7.5 mg. of phosphorus per 100 gm. of body weight) in the form of a 1/10 molar mixture of NaH_2PO_4 and Na_2HPO_4 at pH 7.35 into 49-day-old rachitic rats. Histologically they observed beginning calcification in the cartilage matrix in the majority of the animals killed at the end of 4 hours, and almost uniformly after 8 hours. The deposit of calcium increased in density up to 24 hours. Urist and McLean [5] reported that calcification was initiated in the epiphyseal cartilage within 24 hours after the injection of phosphate solution. Within 48 hours, and following two injections, calcification was usually observed in the osteoid zone in the metaphysis, and in the osteoid borders on the old trabeculae of spongy bone at the junction of the rachitic metaphysis with the shaft. They observed further that shortly after calcification began in the cartilage, orderly penetration and removal of cartilage by advancing capillaries also began.

The effects of phosphates on the dental and alveolar structures have not been reported so far as we are able to ascertain.

Materials and Methods

This study is based on 12 rats † which were placed on a rachitic diet (Steenbock and Black [6]) at the time of weaning (21 days) and given,

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† The animals used in this series of studies were kindly made available by Drs. F. C. McLean and W. Bloom.

beginning at the age of 49 days, single or multiple daily intraperitoneal injections of phosphate solutions. The animals were sacrificed from 6 hours to 6 days following the first injection (Table I).

The standard phosphate solution was a mixture of 80 per cent of 1/10 molar secondary sodium phosphate (M/10 Na_2HPO_4) and 20 per cent of 1/10 molar primary sodium phosphate (M/10 NaH_2PO_4) of which the hydrogen-ion concentration at 38° C. corresponded approximately to pH 7.35. The standard dosage employed was 2.5 cc. (containing approximately 7.5 mg. of phosphorus) per 100 gm. of rat weight. This dose is subtoxic for rachitic rats, rarely resulting in the death of an animal in tetany (McLean and McCoy [4]).

TABLE I

Data on Twelve Albino Rats Placed on a Rachitogenic Diet after Weaning and Treated with Phosphate 1 to 6 Days before Sacrifice

Rat number	No. of injections	Doses of 1/10 molar phosphate solution	Age at death	Duration of experiment
		(*cc.*)	(*days*)	
405	1	1.30	50	1 day
406	2	1.35, 0.67	51	2 days
407	3	1.27, 0.67, 1.27	52	3 days
408	4	1.37, 0.68, 1.37, 1.45	53	4 days
409	5	1.32, 0.66, 1.32, 1.30, 1.30	54	5 days
412	6	1.87, 0.93, 1.87, 2.02, 2.02, 2.02	55	6 days
3805	1	1.05	49	6 hours
3804	1	1.05	50	1 day
3806	2	1.10, 0.85	51	2 days
3807	3	1.15, 1.15, 1.15	52	3 days
3808	4	1.02, 1.02, 1.02, 1.02	53	7 days
3809	5	2.50, 2.50, 2.50, 2.50, 2.50	54	5 days

The heads of the experimental and control rats were removed and fixed in 10 per cent formalin immediately after sacrifice. The jaws were dissected and the upper incisors and molars were prepared for decalcified histologic sections. The incisors were cut in serial longitudinal sections and the molars in serial mesiodistal sections. The stains used were hematoxylin and eosin.

FINDINGS

Alveolar Bone

Lingual Alveolar Plate of the Upper Incisor. In the upper incisor of the normal rat the middle portion of the lingual alveolar bone consists of a plate approximately 0.1 mm. thick, which separates the periodontal membrane from the maxillary sinus (Fig. 1). The periodontal surface of this plate is covered by a continuous row of osteoblasts. Its nasal surface is covered by numerous osteoclasts and is continuously

resorbed, apparently in conjunction with the growth of the maxillary sinus. The alveolar plate is perforated by a few vessels.

In advanced cases of rickets this plate has increased to about double its normal size and consists completely of osteoid tissue. A layer of osteoblasts on its periodontal surface indicates progressing apposition. The nasal surface is covered by spindle-shaped connective tissue cells and is devoid of osteoclasts and Howship's lacunae. This suggests that resorption on this surface has come to a standstill.

No significant changes were observed in this area 24 hours after a single injection of phosphate solution (Fig. 3). Following two daily injections of phosphate, islands of calcification could be observed on the nasal surface (Fig. 2). Adjacent to the calcified areas, the connective tissue cells differentiated into osteoclasts lying in shallow lacunae. Three days later the calcification had almost reached the periodontal surface. Seams of osteoid tissue remained adjacent to the penetrating vessels and to the periodontal surface (Fig. 4).

Fundic Bone of the Incisor. Normally the alveolar bone at the fundus is formed by a cup-shaped lamella approximately 0.05 mm. in thickness (Fig. 5). The bone is continuously resorbed on the surface facing the incisor. At the nasal surface, which is bordered by the mucous glands of the nasal cavity, apposition is in progress. In cases of severe rickets the lamella is twice as thick as is normal and consists entirely of osteoid tissue. The adjacent connective tissue of the periodontal membrane has degenerated and is transformed into hyaline tissue, forming strands and irregular trabeculae. Groups of connective tissue cells are included between the hyaline trabeculae. The border between osteoid and hyaline tissue is indistinct. The hyaline connective tissue is eosinophilic, as is the osteoid tissue.

Six hours after the injection of phosphate the tips of the hyaline trabeculae at the apical periodontal tissue stained with hematoxylin, indicating the beginning of calcification; 18 hours later the area of calcification had markedly widened (Fig. 6). One day later osteoclasts appeared at the periodontal surfaces and between the hyaline trabeculae (Fig. 7). The osteoclasts increased in number during the subsequent days of the experiment (Fig. 8). The hyaline trabeculae were reduced to small islands. They were surrounded by connective tissue which had replaced the hyaline tissue. The osteoclasts were found adjacent to the remnants of hyaline tissue or in the connective tissue. On the fifth day (Fig. 9) the hyaline tissue had almost entirely disappeared. Large osteoclasts had approached the previous border between hyaline and osteoid tissue which appeared to be slightly calcified in its oldest layer. On the sixth day the entire bony lamella had calcified ex-

cept for a thin osteoid seam at the nasal surface. The osteoclasts were attacking the plate proper (Fig. 10).

A similar reaction to the injection of phosphate could be observed in the premaxillary part of the alveolar bone at the convex side. In this region a hyalinization of the alveolar periosteum and compression of the enamel organ are found in severe cases of rickets.[2] Following injections of phosphate, the hyaline tissue calcified, was removed by osteoclasts, and the compressed enamel organ recovered.

Alveolar Bone of the Molars. In cases of severe rickets the lamina dura around the molars is formed by a layer of osteoid tissue. It is thickest at the crest of the septa. The continued apposition of osteoid tissue at the crest of the interradicular septa leads to an obliteration of the periodontal membrane when the vertical eruption of the molars ceases.[2]

Between the third and fifth days after the administration of phosphate the osteoid tissue began to calcify in its deepest layer where it bordered on the pre-experimental calcified core of the septa. The newly calcified bone was not as darkly stained with hematoxylin as was the pre-experimental bone. In the areas close to the periodontal surface the calcification was not even, because the osteoid tissue, in which bundles of Sharpey's fibers are contained, calcifies later than the areas between the bundles.

The obliteration of the periodontal membrane at the bifurcation of the molars began to disappear in some animals on the fourth day of phosphate administration. The width of the osteoid tissue covering the trabeculae of the spongy bone in the alveolar process gradually diminished during the period of phosphate administration.

Dentin of the Upper Incisor

The rachitogenic diet causes not only a retardation in calcification of the dentin but also a marked decrease in dentin formation. This is indicated by the widening of the predentin layer and a decrease in the total width of dentinal wall.[1]

Following phosphate injection, not only was the calcification of the predentin speeded up but also the formation of dentin was considerably accelerated (Table II). The intermediate dentin which disappears while on the rachitogenic diet reappeared after phosphate administration, just as was observed after administration of vitamin D or after fasting.[7] It was found not only in the middle third of the enamel-covered dentin, as in normal animals, but extended into the incisal part of the basal third. The basal predentin of the incisor which is entirely

uncalcified in rachitic animals calcified during the first 3 days of phosphate administration.

Discussion

McLean and McCoy [4] established that the epiphyseal cartilage of rachitic rats begins to calcify as early as 4 hours after injection of phosphate. Calcification of the osteoid tissue at the alveolar process could not be seen in our material until the end of the second day following phosphate administration. The first layers to calcify were the oldest layers of the osteoid tissue. Resorption of the calcified osteoid tissue by osteoclastic activity occurred at a slow rate.

The hyaline tissue which was formed by the degeneration of the compressed connective tissue differed markedly in its behavior from that of osteoid tissue, although microscopically these two tissues appeared very similar. The hyaline tissue showed signs of calcification, almost simultaneously with the epiphyseal cartilage. The layers of

Table II

Width in Microns of Predentin, Calcified Dentin, and Dentinal Labial Wall of Rachitic Rats Given Intraperitoneal Injections of Phosphate*

Rat no.	Duration of experiment	Predentin	Calcified dentin	Labial dentinal wall
	(*days*)	(μ)	(μ)	(μ)
3805	1	61.6	31.3	92.9
3804	2	49.7	68.1	117.8
3806	3	47.8	75.4	123.2
3807	4	41.4	83.7	125.1
3808	5	34.0	100.3	134.3
3809	6	34.0	118.7	152.7

* Measured at the level of completed formation of enamel matrix and representing an average of three measurements.

hyaline tissue which had been formed *last* were the first to calcify. After calcification the hyalin was speedily removed by osteoclastic resorption. These observations show biologic differences in the nature of the osteoid and the hyaline tissues.[2]

The disappearance of the compression of the periodontal membrane between interradicular septa and molars was in all probability due to the resumption of the endochondral growth in the mandibular condyle. The distance between maxillary and mandibular bodies was again increased and space was opened for the vertical eruption of the molars.

The action of phosphates on the dentin was identical with that of vitamin D and fasting,[7] both of which induce a rise in the level of the inorganic phosphorus of the blood.

The findings in this study and by previous investigations show that

intraperitoneal injections of phosphate lead to a healing of rickets in the absence of vitamin D. These evidences support the assumption that one rôle of vitamin D is the increase of the phosphorus level of the blood.

Summary

The effect of intraperitoneal injections of phosphate on the alveolar bone and the dentin was studied histologically in 12 rachitic rats. The findings were:

1. Osteoid tissue begins to calcify at the end of the second day after the administration of phosphate; the calcification begins in the oldest layers of the osteoid tissue.

2. The hyaline tissue formed by the degeneration of connective tissue shows the first signs of calcification almost immediately after injection of phosphate; the first layers to calcify are those which have been formed last.

3. After calcification of osteoid and hyaline tissues, osteoclasts reappear and resorb the calcified tissues; the resorption of calcified hyaline tissue progresses at a faster rate than that of calcified osteoid tissue.

4. Beginning with the fifth day of the experiment the encroachment of the periodontal membrane between molars and interradicular septa disappeared. This is probably caused by the resumption of the vertical eruption of the molar correlated with the resumption of condylar mandibular growth.

5. The dentin shows a resumption of normal calcification and a normal rate of formation.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 186

Photomicrographs of the lingual alveolar bone. The area is indicated in the insert in Figure 1. Ob. = osteoblasts; Oc. = osteoclasts; P.d.m. = periodontal membrane; Sm. = submucosa; V.s. = vessels penetrating the bony lamella. × 214.

FIG. 1. Rat 3801, 54 days of age, placed on normal basic diet. The normally calcified bony lamella is seen. Osteoblasts facing the incisor indicate progressing apposition; osteoclasts on the side of the nasal cavity indicate resorption.

FIG. 2. Rat 3806, 51 days of age, placed on rachitogenic diet for 30 days and sacrificed 2 days after two intraperitoneal injections of phosphate. At the nasal surface of the bony plate beginning calcification and resorption are seen.

FIG. 3. Rat 3804, 50 days of age, placed on rachitogenic diet for 29 days and sacrificed 24 hours after a single intraperitoneal injection of phosphate. The bony lamella is about twice as thick as normal, consisting entirely of osteoid tissue. Osteoblasts indicate progressive apposition.

FIG. 4. Rat 3809, 54 days of age, placed on rachitogenic diet for 33 days and sacrificed 5 days after five intraperitoneal injections of phosphate. Calcification is in an advanced stage. The osteoid layer persists next to the periodontal surface and penetrating vessels.

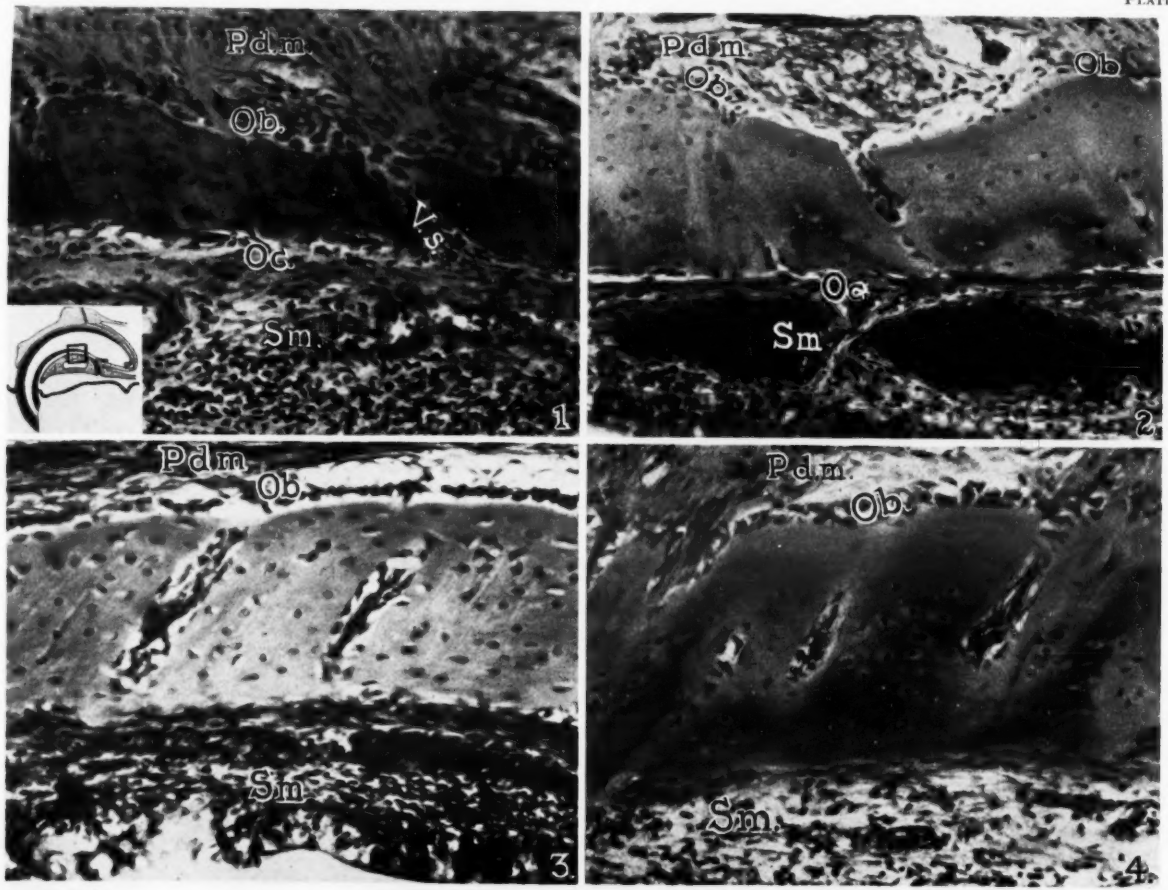


PLATE 187

Photomicrographs of the fundic bone. The area is indicated in the insert in Figure 5. P.d.m. = periodontal membrane; Mg = mucous glands of the nasal cavity. × 233.

FIG. 5. Rat 3801 placed on normal basal diet. The normally calcified bony lamella shows bone apposition at the nasal surface and resorption at the periodontal surface.

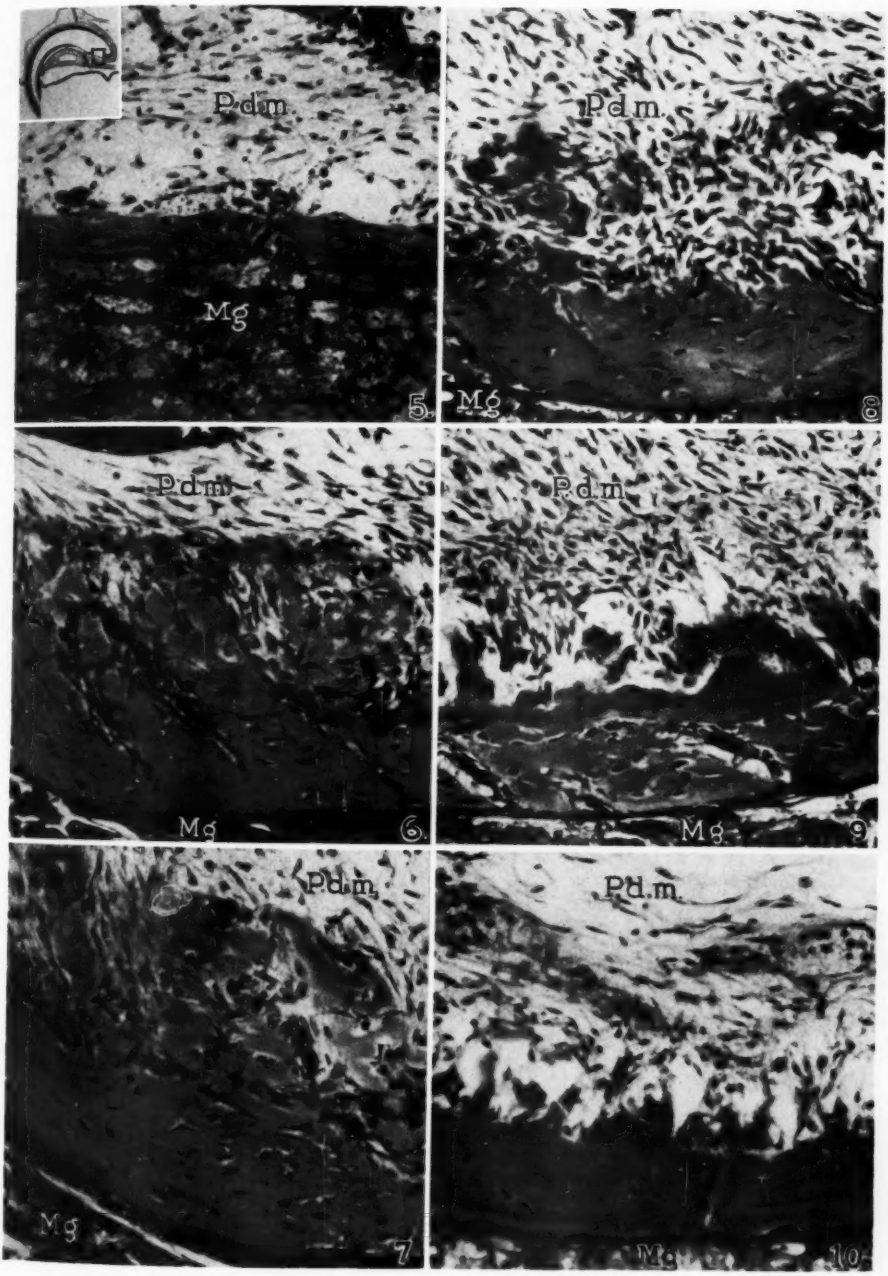
FIG. 6. Rat 405, 50 days of age, placed on rachitogenic diet for 29 days and sacrificed 24 hours after a single intraperitoneal injection of phosphate. The bony lamella is about twice as thick as normal, consisting entirely of osteoid tissue, and a large portion of the periodontal membrane is degenerated and transformed into hyalin. The tips of hyaline trabeculae are stained with hematoxylin, indicating beginning of calcification.

FIG. 7. Rat 406, 51 days of age, placed on rachitogenic diet for 30 days and sacrificed after two intraperitoneal injections of phosphate. Osteoclasts appear between and at the periodontal surfaces of hyaline trabeculae.

FIG. 8. Rat 408, 53 days of age, placed on rachitogenic diet for 32 days and sacrificed after four intraperitoneal injections of phosphate. The fibrous tissue contains numerous osteoclasts which have resorbed hyaline trabeculae, leaving only small strands of hyaline tissue.

FIG. 9. Rat 409, 54 days of age, placed on rachitogenic diet for 33 days and sacrificed after five intraperitoneal injections of phosphate. Fibrous tissue has replaced hyaline trabeculae almost completely and osteoclasts are bordering the fundic bone at the periodontal side.

FIG. 10. Rat 412, 55 days of age, placed on rachitogenic diet for 34 days and sacrificed after six intraperitoneal injections of phosphate. Osteoclasts are numerous at the periodontal surface of the fundic bone, which is stained with hematoxylin, indicating calcification, except for an osteoid layer at the nasal surface.



Weinmann and Schour

Effect of Phosphate

INTESTINAL LIPODYSTROPHY (WHIPPLE'S DISEASE) *

PATRICK J. FITZGERALD, M.D., and THOMAS D. KINNEY, M.D.
(From the Mallory Institute of Pathology, Boston City Hospital, Boston 18, Mass.)

In 1907 Whipple [1] described for the first time a disease entity for which he suggested the term "intestinal lipodystrophy." His report was based on the case of a 37-year-old physician who had a 5-year history of progressive asthenia and weight loss, developed diarrhea and fatty stools a few months before death, and showed a striking lipoid deposition in the mesentery and small intestine. Since that time 6 additional cases have been reported which show the characteristic lesions described by Whipple. The present case is the eighth case to be reported.

Also of interest in this case are the bizarre hematologic findings that led to a diagnosis of atypical lymphatic leukemia, treated by radioactive phosphorus; and a subsequent diagnosis of hemolytic icterus, treated by splenectomy.

After Whipple's description in 1907, the next report of similar cases was that of Blumgart [2] (1923) who described 3 cases of progressive emaciation associated with steatorrhea. Jarco [3] (1936) reported a case similar to Whipple's which he stated was the third such case to be reported, since Jarco and others have accepted only one of Blumgart's cases (case 2) as belonging to this disease entity.

Reinhart and Wilson [4] (1939) and Sailer and McGann [5] (1942) have described cases which presented a histologic picture similar to that described by Whipple.[1] Sailer and McGann, in their review of the subject, included 4 cases (Fleischmann,[6] Bargen, Bollman, and Kepler,[7] Korsch,[8] Hill [9]) which they believed showed the characteristic syndrome. Sailer and McGann termed the disease lipophagic granulomatosis. Of the cases listed, only one (Korsch) seems to have had the characteristic changes of mesenteric nodes and bowel.

The most recent case is that of Pearse.[10] His patient related a 2-year history of recurring bouts of abdominal distention, intermittent crampy pain, and diffuse tenderness of the abdomen. At operation cysts filled with lipid were found in the peritoneum, omentum, intestines, and liver. Whipple personally examined the histologic sections of these lesions and stated that the lesions were similar to those he had originally described. Pearse's report does not mention mesenteric lymph nodes and no microscopic examination of the small bowel was made. This was the first diagnosis made ante-mortem and the first case to show changes in locations other than the abdominal nodes or small bowel.

* Received for publication, January 22, 1945.

Report of Case

The patient (B. C. H. no. 1,080,279), an Armenian male, 48 years old, entered the hospital on September 10, 1942, because of persistent diarrhea for 1 month. He had been followed in various Boston hospitals since June, 1941, when he entered a local hospital because of weakness, night sweats, and cough. At that time the spleen was felt 6 cm. below the left costal margin and extended to the mid-abdominal line. The hemoglobin was 11 gm. (Sahli); the red blood cell count, 4,630,000; hematocrit, 37; white blood cell count, 41,000, of which 80 per cent were large lymphocytes considered abnormal and unclassified. Oxidase stain 6 days later showed 61 per cent of the leukocytes to be oxidase-negative. The erythrocyte fragility test was considered to be within normal limits. The bleeding time was 1 minute; the clotting time, 10 minutes. The nonprotein nitrogen was 33; the total protein, 6.6; albumin, 4.5; globulin, 2.1 mg. per 100 cc. The basal metabolic rate was +24. Microscopic examination of a lymph node secured for biopsy showed well differentiated, moderately active germinal centers which were not anaplastic. There was no evidence of leukemia or neoplasm in this node. A diagnosis of hyperplasia was made. A specimen of the sternal marrow was interpreted as "hyperplasia of bone marrow." The clinical diagnosis was lymphatic leukemia with atypical cells. The patient was treated with radioactive phosphorus.

He was seen at this same hospital 7 months later because of persistence of weakness and cough. At that time he was slightly icteric; the spleen was down to the left iliac crest and extended across the midline. The liver for the first time was percussed 2 fingersbreadth below the right costal margin. The hemoglobin was 5.4 gm.; red blood cell count, 1,380,000; hematocrit, 18; platelets, 140,000 per cmm. The white blood cell count was 19,600 and 70 per cent of the white cells were lymphocytes, which were thought to be similar to those seen when the blood was first studied. A specimen of the sternal marrow again was interpreted as showing "hyperplasia of the marrow." The icteric index was 21. The patient was given multiple transfusions and was instructed to take iron and brewer's yeast.

On April 6, 1942, because of weakness, palpitation, dizziness, dyspnea, and occasional mild anterior chest pain, he was admitted to the II Medical (Harvard) Service of the Boston City Hospital. At the time of admission the temperature was 99° F.; pulse, 100; respirations, 24; blood pressure, 110/60 mg. Hg. He was a normally developed but poorly nourished, middle-aged Armenian who appeared to be chronically ill. There was moderate exophthalmos. Examination of the fundi showed multiple small, hemorrhagic areas, most marked about the vessels. The neck veins were distended. There were scattered moist râles at the lung bases. The heart was enlarged to the left, being in the anterior axillary line in the sixth interspace. There was a blowing systolic murmur over the entire precordium, but most marked at the apex. P_2 was louder than A_2. The liver edge was smooth and was felt well below the level of the umbilicus. The spleen was enlarged, smooth, and firm, and was also felt below the level of the umbilicus.

At the time of entry the hemoglobin * was 21 per cent (Sahli); red blood cell count, 750,000; white blood cell count, 34,000. The differential blood count gave polymorphonuclear leukocytes, 21 per cent; band forms, 5 per cent; eosinophils, 1 per cent; small lymphocytes, 15 per cent; large lymphocytes, 45 per cent; atypical lymphocytes, 9 per cent; monocytes, 2.5 per cent; metamyelocytes, 1 per cent; myelocytes, 0.5 per cent. There were 25 to 36 per cent reticulocytes, and the red blood cells showed variation in size with spherocytes and many large polychromatophilic cells. The hematocrit reading was 10; icteric index, 50 to 75; van den Bergh reaction (direct), 0.9 mg. per cent, (indirect) 7.6; D/I, 12 per cent. The

* The authors wish to thank Miss Geneva Darland for the hematological studies in this case.

blood platelet count was 73,000. Prothrombin was 100 per cent. The bleeding time was 3.5 minutes. The blood coagulation time was 5.5 minutes in glass and 11.5 minutes in lusteroid. The nonprotein nitrogen was 24 mg. per 100 cc. The total proteins were 5.12 mg. per 100 cc. The results of urine and stool examinations were negative.

Erythrocytic fragility as determined by resistance to hypotonic saline solution showed, on three successive examinations over a period of 3 weeks: 1 per cent hemolysis of the red cells in dilutions of 0.84 to 0.77; 10 per cent hemolysis at 0.67 to 0.61; 50 per cent hemolysis at 0.50 to 0.45; and 75 per cent hemolysis from 0.44 to 0.39. A normal control showed 1 per cent hemolysis at 0.45; 10 per cent at 0.41; 50 per cent at 0.38; and 75 per cent at 0.36 dilution. The acid hemolysis test, Donath-Landsteiner test, test for hemolysins, and test for cold agglutinins were all negative.

Three brothers, two sons, and a daughter of the patient showed normal fragility curves. It was believed that the patient was suffering from hemolytic jaundice and on May 1, 1942, a splenectomy was performed.

The spleen weighed 635 gm. and measured 26.3 by 16.3 by 5 cm. There were a few scattered fibrous adhesions over the capsular surface. Five slightly raised, irregular, yellow areas of infarction were present and these varied in size from 1 to 5 cm. in their greatest diameters. The remaining surface was smooth and dull red-purple-gray. There was a deep notch at each pole of the spleen. The pedicle measured 2 cm. in width. The splenic artery and vein were patent. The organ was firm. The cut surfaces were purple-gray and were discolored by the areas of infarction. The large blood vessels were prominent as were the trabeculations. The follicles could not be identified on the homogeneous purple surface.

Microscopic examination of sections of the spleen showed areas of infarction with adjacent areas of hemorrhage. Throughout the spleen there was intense engorgement of the pulp with compression of the follicles. Large spaces were lined with hypertrophied endothelial cells. There were deposits of hemosiderin scattered throughout the pulp. The follicles showed the usual structure.

Seven days after operation, the patient's erythrocytic fragility had decreased to 1 per cent hemolysis at 0.54 dilution, 75 per cent at 0.35. However, 1 month following operation 1 per cent hemolysis occurred at 0.70, and 75 per cent at 0.46, and the fragility remained near this level during the remainder of the hospital stay.

The week following operation the patient began to suffer from soreness and colicky pain in the abdomen and from anorexia without nausea or vomiting. The temperature was 100° F.; pulse, 88; respirations, 20. These symptoms continued and in addition the patient developed signs of infarction of the lower lobe of the right lung. A roentgenogram of the chest was consistent with this diagnosis. A gastrointestinal series was negative. A Graham series showing the gallbladder to be poorly filled by dye was considered to be evidence of disease of that organ. During the eleventh hospital week the patient's temperature reached 103° F. on two occasions and fell by lysis to normal in 7 days. At this time there was much abdominal distention with a slight increase in icterus, and a palpable, tender mass measuring approximately 5 cm. was found in the left upper quadrant. After 13 days of sulfadiazine administration the temperature fell to normal and remained

so thereafter. He was considered clinically to have had a subdiaphragmatic abscess.

It should be noted that 2½ months following operation the hemoglobin was 75 per cent; the red blood cell count, 3,360,000; the hematocrit, 33.8; and the white blood cell count 22,400, with the following differential count: polymorphonuclear leukocytes, 40.5 per cent; band forms, 6 per cent; basophils, 2 per cent; small lymphocytes, 8 per cent; monocytes, 7 per cent; and metamyelocytes, 0.5 per cent.

The patient was discharged on August 5, 1942, apparently well, to be followed in the out-patient clinic, but was readmitted to the hospital on November 22, 1942, because of diarrhea of 1 month's duration. Bowel movements varied from one to seven per day, the stools being green to brown and occasionally containing mucus but no blood. The patient complained also of epigastric pain, increasing dyspnea, weakness, and swelling of the ankles for the same period of time. The temperature was then 98.6° F.; pulse, 102; respirations, 22; blood pressure, 95/50. The heart was moderately enlarged to the left. There were dullness and decreased breath sounds over the bases of both lungs. The liver edge was tender and was felt 4 fingersbreath below the costal margin in the right midclavicular line. There was pitting edema of the lower extremities.

The patient was treated symptomatically. In addition, he was given enteric-coated sulfadiazine tablets, and a course of mercupurin with resulting diuresis and disappearance of ankle edema. The diarrhea persisted, with only occasional scattered days of freedom. After 2 months of unsuccessful hospitalization he became restless and left the hospital against the advice of the physicians.

Twenty-four hours after leaving the hospital the patient died suddenly, unattended by a physician.

During the final hospital admission the following laboratory data were secured: hemoglobin, 81 per cent (Sahli); red blood cell count, 3,800,000 per cmm.; hematocrit, 38.6 per cent; mean corpuscular volume, 101.6 cu. μ; mean corpuscular hemoglobin concentration, 32.7 per cent; mean corpuscular hemoglobin, 33.3 micromicrograms; reticulocytes, 2.9 per cent; icteric index, 5; white blood cells, 13,500 per cmm.; differential count: polymorphonuclear neutrophils, 45.5 per cent; polymorphonuclear neutrophils (band forms), 27.0 per cent; eosinophils, 1 per cent; basophils, 0.5 per cent; small lymphocytes, 9.5 per cent; large lymphocytes, 4 per cent; adult monocytes, 7 per cent; young monocytes, 3 per cent.

AUTOPSY FINDINGS

The body (M.I.P. no. A42-709) was that of a normally developed, emaciated, white male. The skin was sallow with slightly increased pigmentation consistent with the patient's Armenian lineage, but no icterus could be demonstrated. The body cavities were not remarkable. The heart and lungs were negative. The spleen was absent but a splenculus, 2 cm. in diameter, was found which presented the usual splenic structure. The liver was markedly enlarged and extended across the upper abdomen to the left midclavicular line. It extended 10 cm. beneath the costal margin in the right midclavicular line and 15 cm. beneath the tip of the xiphoid. The gallbladder was not remarkable and contained 50 cc. of thin yellow-green fluid. The esophagus and stomach were grossly negative. The entire small bowel was dilated to twice its usual size. The lumen contained a moderate quantity of liquid, gray-white, fecal material. The wall of the small intestine was

2 or 3 times its usual thickness (Fig. 1). This increase appeared to be due solely to changes in the mucosa which was filled by a white, fatty substance resulting in countless granular, friable, frond-like projections. The rugae were prominent. When the mucosa was cut it was possible to express this white, fatty substance. The Peyer's patches were not grossly discernible. The muscle layers and serosa of the small bowel were not remarkable. The lesion ended abruptly at the ileocecal valve and no abnormalities were present in the large bowel.

There were many discrete, firm, gray-white, enlarged mesenteric nodes which averaged 2 cm. in diameter. The cut surfaces of the nodes were pale gray with a few pinhead-sized, discrete nodules scattered throughout.

The kidneys weighed 340 gm. There was a 2.5 cm. shallow depression with a firm, gray-yellow center and thin, dark purple periphery at the lower pole of the right kidney. Adjacent to this area were 15 to 20 small areas of purple discoloration varying from 0.05 to 0.5 cm. in diameter.

Gross examination of the remainder of the organs showed nothing of note.

Microscopic Examination

The esophagus, stomach, and duodenum were negative. Identical histologic lesions were present in the jejunum and ileum. The villi varied greatly in size (Fig. 2), some being three or four times the usual width, while slender villi were frequently five or six times the usual length. This increase in size was associated with the presence of a lipoid substance or substances in three physical forms. The first was a homogeneous, lightly stained, fine coagulum which took a pale pink stain with phloxine, and distended the lacteals of the enlarged villi (Fig. 3). The second form appeared as vacuoles of various sizes scattered throughout the coagulum and also making up a small part of the area of the villus. The third consisted of accumulations of large mononuclear cells with vacuolated or foamy cytoplasm scattered throughout the villi. The tunica propria, present at the periphery of the dilated villi, was thickened, and there was increased cellularity. There were large, dilated spaces throughout the submucosa containing the same elements noted in the villi. The muscularis and serosa were normal at low power magnification.

At higher magnification the large spaces in the submucosa containing the pink coagulum were seen to be lined by endothelial cells. Some of these endothelial cells were enlarged and contained small vacuoles in the cytoplasm. The presumption was that these large spaces were lymphatic sinusoids. A majority of the sinusoids contained many mononuclear cells which varied in size from that of the usual phagocyte

to gigantic "foam" cells eight to ten times the diameter of the former (Fig. 4). These large cells had an indistinct, small nucleus and very large, multivacuolated, frothy cytoplasm. They commonly occurred about the periphery of the dilated lymphatics, and in many villi they seemed to have infiltrated into the lipoid substance and to be phagocytizing it. In some sinusoids they occupied most of the area, but generally they made up a small portion of the substance. Multinucleated giant cells were occasionally seen and these had foamy cytoplasm. Also enmeshed in the lipoid material of the villi were a few lymphocytes and plasma cells. The interstices of the displaced tunica propria showed considerable pink coagulum, vacuolization, and infiltration with mononuclear cells with the foamy cytoplasm. There was an increase in the number of lymphocytes, plasma cells, and connective tissue in the tunica.

The epithelium of the mucous membrane of the villi was intact and only a rare cell contained a few vacuoles in its cytoplasm. The cells of the crypts of Lieberkühn were similarly free of change, as were the goblet cells and the lymphoid follicles.

The stroma of the mucous membrane was distended by many large, clear vacuoles and much pink coagulum, and there were increased eosinophils, plasma cells, and lymphocytes present. Diffusely scattered throughout the stroma were many large mononuclear cells with frothy cytoplasm similar to those seen in the villi. In some sections, particularly those stained for fat, the lipoid could be seen distending the villi and extending throughout the tunica and muscularis to branch off sharply at right angles in the submucosa and run parallel with the muscular layers, nicely outlining the lymphatic pattern.

The muscular layers showed only scattered foci of a few lipoid-bearing cells. These were more common in the connective tissue between the circular and longitudinal muscles. In the serosa there were a few areas in which a moderate number of mononuclear cells and an occasional large area contained material similar to that in the villi, giving a positive reaction to the fat stain. The endothelial cells of a blood vessel occasionally contained vacuolated cytoplasm, also giving a positive reaction for fat.

The large intestine was negative.

When the jejunum and ileum were stained with sudan IV the lipoid material became bright red. These lipoids were pink-red with Nile blue sulfate (neutral fats). Polariscopic examination showed a few small, doubly refractile pinpoints scattered throughout the villi, mucosa, and submucosa. Most of the lipoid substance, however, was nonrefractile and no more than 10 per cent could be considered to be anisotropic.

Chemical analysis of a section of small intestine * showed the fatty substance in the analyzed specimen to be mainly phospholipids and neutral fat. The cholesterol was only slightly increased (Table I).

As a result of differential staining, polariscopic examination, and chemical analysis, the lipoid material was considered to be a mixture of both phospholipid and neutral fat with possibly a very slight increase in cholesterol. Neutral fat predominated as evidenced by the Nile blue sulfate stain and chemical analysis.

The cortical and medullary sinuses of the mesenteric lymph nodes were dilated to four or five times their usual size and filled with a pale, homogeneous coagulum similar to that seen in the villi (Fig. 5). There was crowding and thinning out of the follicles. As in the villi, the coagulum was accompanied by many mononuclear cells with foamy

TABLE I

Results of Analysis of Small Intestine

Wet weight of tissue used	3.2 gm.
Dry weight of tissue used	0.4886 gm
	Mg. per 100 mg. dried tissue
Total cholesterol	3.16
Total phospholipids	14.95
Total fatty acids	19.9

cytoplasm varying in size from the usual phagocyte to cells eight to ten times the diameter of the former (Fig. 6). There was moderate increase in trabecular connective tissue. Phagocytes which had engulfed hemosiderin were found in all nodes examined.

In many of the nodes, huge mononuclear cells with frothy cytoplasm and vesicular nuclei replaced the coagulum in the dilated sinusoids. Scattered throughout sinusoids of all the nodes were many lymphocytes, plasma cells, and mononuclear cells. Many of the endothelial cells lining the sinusoids were swollen and had a vacuolated cytoplasm. Occasionally multinucleated giant cells were found.

In the lymph nodes many cells contained circular, discrete, acidophilic intranuclear inclusions that had at their periphery a clear, thin zone which was surrounded by fragmented nuclear chromatin. Rarely a similar inclusion was present in the cytoplasm of a cell. The intranuclear inclusions were found in plasma cells and in the nuclei of both the large and small macrophages which contained lipid. While the lipid in the coagulum and cytoplasm gave a positive stain for fat and was apparently the same as the fat in the small bowel, none of the nuclear

* We are indebted to Dr. S. J. Tannhauser for the analysis of this specimen.

or cytoplasmic inclusions gave a positive stain for fat and under the polariscope they were not anisotropic.

A large lymph node taken from the junction of the cystic and common ducts showed similar changes but to a lesser degree. The lymphoid sinuses were dilated to about twice their usual width and contained many phagocytes with foamy cytoplasm. There was only a small amount of coagulum present and there was only a slight encroachment upon the lymphoid elements. There was a slight increase of trabecular connective tissue throughout.

There was much granular, black pigment present in the tracheal nodes together with fibrosis. Phagocytes with foamy cytoplasm were occasionally found.

Microscopic sections of the heart were not remarkable. Alternating areas of atelectasis and emphysema were present in the lungs, and there were foci of alveoli filled with edema fluid and red blood cells.

Microscopic examination of the splenculus showed the usual splenic structure. The changes found in the spleen were not present.

The liver structure was well preserved. The sinusoids were slightly dilated by red cells. There were scattered foci of collagen surrounded by lymphocytes, plasma cells, and large mononuclear cells with vesicular nuclei and large, pale pink, homogeneous cytoplasm. Occasionally, fibroblasts could be identified in the centers of these lesions which frequently, for the most part, were periportal in distribution but were occasionally found in midzonal or pericentral areas. Many liver cells contained hemosiderin and frequently this substance was present in the large mononuclear cells in the granulomatous lesions.

A small area of old infarction was present in the cortex of the kidney. The glomeruli, tubules, interstitial tissue, and pelvis were negative except for small granules of hemosiderin in the epithelium of many of the collecting tubules.

There was a large focus of necrosis in the medulla of the adrenal, surrounded by phagocytes with vacuolated cytoplasm, lymphocytes, and plasma cells. In the cortex were a few areas of collagen in which were seen phagocytes, and there was one large area in the cortex that was composed entirely of huge phagocytes with a foamy cytoplasm.

The bone marrow was very cellular, with hyperplasia and maturation of both the erythrocytic and granulocytic series. Megakaryocytes were present in usual numbers. A few intranuclear inclusion bodies in plasma cells, similar to those seen in the mesenteric nodes, were present. There were large phagocytes containing hemosiderin. There was no evidence of leukemia.

Anatomical Diagnoses. Intestinal lipodystrophy (Whipple's dis-

ease); focal granulomatous lesions of the liver; intranuclear inclusion bodies in plasma cells, cause unknown; focal necrosis of the adrenal; healed infarct of kidney; surgical absence of spleen; healed left rectus incision.

Comment

The blood findings in this case were bizarre. A persistent leukocytosis of 20,000 to 50,000 white blood cells per cmm. was present for months during the early part of the patient's illness. There was relative and absolute lymphocytosis with 70 to 80 per cent of the white cells composed of "large," "atypical," or "abnormal" cells of the lymphoid series. Most of these cells were large with a clear, light blue cytoplasm. It was noted that many of these cells had nuclei of a "young" type and nucleoli were sometimes present.

Following splenectomy, the absolute as well as the relative number of lymphocytes decreased to within normal limits and remained normal for approximately 1 month. The lymphocytes appeared normal. During this period there were many toxic polymorphonuclear neutrophils and many young forms of polymorphonuclear cells. The number of lymphocytes gradually increased to 30,000 and ranged between 15,000 and 20,000 during the last month of life. During this period the cells could not be classified. In general, the cells were younger than when first seen, as indicated by the deep blue of the cytoplasm of many of them. Some of these were true blasts with nucleoli; some resembled young lymphocytes and some young monocytes. The platelets were maintained at a higher level following splenectomy.

At autopsy no anatomical evidence for a diagnosis of lymphatic leukemia was found. Reinhart and Wilson [4] described a "benign lymphocytosis" in their case with a white cell count varying from 15,000 to 20,000, but gave no further data regarding the blood picture. The concomitant occurrence of lymphocytosis and acute hemolytic jaundice is unusual. Acute hemolytic anemia is frequently accompanied by leukocytosis, but it is generally of the myeloid series. Lymphocytosis is stated to occur in chronic diseases where there is a stimulation to reticular hyperplasia such as in lues or tuberculosis. No evidence of any of these infectious diseases was found in our case. It was felt that in the present case the blood showed an unusual type of lymphocytic or histiocytic response of unknown causation.

The erythrocytes were described as showing "normal fragility" in the early part of 1942 at another hospital, but in March, 1942, when studied at the Thorndike Memorial Laboratory of the Boston City Hospital, an increased fragility to the action of hypotonic saline was demonstrated. Other findings consistent with hemolytic jaundice, such

as increased urobilinogenuria, hyperbilirubinemia, splenomegaly, anemia, spherocytosis, and reticulocytosis (27 per cent), were found. Histologic examination of the excised spleen showed findings consistent with hemolytic jaundice. Three brothers, two sisters, and one daughter of the patient all had normal erythrocytic fragility curves.

The anemia which occurs in Whipple's disease has never been adequately explained. It is usually described as "hypochromic" or "secondary." The present case is the only case on record in which fragility studies were made. In this connection it is of interest to consider the effects of lipoid substances upon hemolysis of red blood cells. Haden,[11] Castle and Daland,[12] and others have shown that sphericity of the red blood cells is associated with increased fragility. Tompkins [13] was able, by repeated intravenous injections of lecithin, to produce in rabbits a slowly progressive hemolytic anemia associated with a slight increase in hypotonic fragility and a slight tendency of the erythrocytes towards sphericity. Ponder [14,15] has shown that erythrocytes become spheroidal in suspensions of lecithin. There is some experimental evidence which supports the thesis that phospholipids in circulation are adsorbed on erythrocytes. Bloor [16] and Freeman and Johnson [17] have shown the presence of a hemolytic agent in lymph from the thoracic duct during the adsorption of ingested fat. The hemolytic agent responsible is probably a combination of free fatty acid and soap. In view of the fact that the above various lipoid substances have been shown to have a hemolytic action, it would seem that study of the hemolytic action of the lipoid substance found in Whipple's disease would be indicated, since it is possible that such studies might lead to the explanation of the anemia in these cases.

Eosinophilic inclusion bodies were seen in the nuclei of plasma cells in the mesenteric nodes. These were quite striking and have not been noted before in this laboratory, nor have they been described before in this disease.

Discussion

The average age for this group of patients is 49 years, the youngest reported being 36 years of age, while the oldest was 74 years. The greater number occurred in the third and fourth decades. Seven of the 8 cases occurred in males.

The usual symptoms are progressive loss of weight and strength terminating in marked emaciation and diarrhea. Five of the 8 patients had suffered from diarrhea for periods varying from a "brief episode" to 2 years, with this symptom in a majority of the cases persisting for a few months before demise. In one case [4] "constipation" was reported. The stools have been described generally as light yellow, creamy, or

clay-colored except for one case in which the stools were dark brown to black and were guaiac-positive. Some stools have contained bile while others were bile-free. Chemical analyses of stools from 2 cases are available. In Whipple's case [1] 80 per cent of the dried stool was fat, of which 50 per cent was neutral fat and 30 per cent fatty acids. In the case reported by Pearse,[10] 60 per cent of the dried stool was fat, of which 44 per cent was fatty acids, 12 per cent neutral fat, and 3 per cent was unsaponifiable. These results should be compared with Fowweather's [18] figures for normal stools in which 17.5 per cent of the dried stool is fat, of which 7.31 per cent is neutral fat, 4.6 per cent soap fat, and 5.64 per cent free fatty acids. After treatment of Pearse's patient the lipoid content of dry matter decreased from 60 per cent to 25 per cent and only fatty acids were present. Thus in both cases the total fat of the stools was increased; in one case fatty acids were in excess and in the other neutral fats predominated.

No parasites nor ova were seen in any case, nor were any significant organisms cultured from the stools.

In those cases in which agglutination tests for the typhoid, paratyphoid, and dysentery groups were done, the results were negative.

Patients have complained of abdominal distention, vague discomfort, particularly over the upper abdomen, crampy pains over any portion of the abdomen, and even colicky right upper quadrant pains suggesting acute cholecystitis. In 5 of the reported cases there was a long history of polyarthritis varying from 5 to 15 years, and in 4 of 7 cases in which the thorax was examined there was an organizing or organized pericarditis which was described as "obliterative" or adhesive; 3 showed pleural adhesions and 3 revealed healed verrucous endocarditis.

Progressive anemia generally described as "secondary" has been described in most cases. One case (Blumgart [2]) had a color index greater than 1 and ours showed increased fragility consistent with a diagnosis of hemolytic anemia. White, differential, and reticulocyte counts have been within normal limits except for the present case and one other discussed above. The urine was negative in all cases.

In 5 cases a slight, yellow, generalized pigmentation of the skin was noted. Interstitial fibrosis of the pancreas was described in 2 of the 7 autopsied cases. In 1 case a granulomatous lesion was present in the liver which was similar to that described in the present case.

The pathologic findings in Whipple's disease are characterized by deposits of fat and fatty acids in the small intestine and mesenteric lymph nodes. Grossly, the mucosa of the jejunum and ileum is swollen and is flecked with minute deposits of yellow-white lipoid substance. The severity of the lesion may vary, the mucosa in one case being pink

flecked by yellow, while in other cases the deposits are so great as apparently to involve the entire mucosa and give to it a definite yellow color. The mucosa is not ulcerated and the Peyer's patches are not unduly prominent. Microscopic examination of the bowel wall shows large deposits of lipoid substance in the villi, submucosa, and the interglandular tissue of the mucosa and submucosa. The villi are double the usual length and breadth and are filled with lipoid substance. These areas also show large spaces lined with endothelial cells which suggest dilated lymph radicles. Lipoid substance completely fills many of these spaces. Scattered throughout the villi, submucosa and mucosa are many mononuclear cells whose cytoplasm is vacuolated. A few multinucleated giant cells containing vacuoles are also present. Deposits of lipoid are found in the endothelial cells lining the capillaries. The lymph follicles are not involved to any striking degree, containing but an occasional fat-laden monocyte. The lipoid substance takes the sudan IV stain well, the coagulum, vacuoles and foamy cytoplasm taking a bright red stain. These substances stain pink-red with the Nile blue sulfate stain which is considered suggestive of neutral fat. Polariscopic examination shows most of the substance to be non-doubly refractile. Chemical analysis indicates that the lipoid substance is largely phospholipids and neutral fat. Whipple's analysis showed the neutral fat to have a low saponification number, which he interpreted as indicating either some abnormality of the fat or the presence of some nonsaponifiable substance mixed with it.

The mesenteric and occasionally the retroperitoneal and peripancreatic lymph nodes are enlarged and their cut surfaces are pale yellow. Microscopic sections of the nodes show that the cortical and medullary sinuses are markedly dilated and are filled with lipoid substance similar to that noted in the small intestine. In many nodes the follicles have been compressed or have disappeared. As in the intestine, there are many mononuclear cells containing the lipoid substance. Other sinuses are filled with huge, lipid-laden mononuclear cells.

The nature of the changes in the joints in Whipple's disease is unknown as the joints were not studied in any of the cases reported.

The cause of Whipple's disease is unknown. Obstruction of the thoracic duct or mesenteric lymph vessels comes naturally to mind. However, in cases of chylous ascites due to obstruction of the thoracic duct by various causes no enlargement of mesenteric nodes nor intestinal lesions such as are described in cases of Whipple's disease occur. Also, Karoliny [19] was unable to reproduce the lesions by ligation or compression of the thoracic duct in animals. Furthermore, no evidence

of occlusion of the lymph passages or thoracic duct has been found in any of the reported cases of this disease.

The clinical course is not unlike that of nontropical sprue. However, it has never been demonstrated that any characteristic pathologic lesions occur in the intestines in sprue.[20] Certainly, no lesions have ever been demonstrated in sprue that resemble those of Whipple's disease.

Idiopathic intestinal lipodystrophy does not fit into any of the known diseases of lipoid storage such as Niemann-Pick's disease, Hand-Schüller-Christian's disease, or Gaucher's disease. All anatomical, clinical, and chemical evidence serves to exclude Whipple's disease from this group. Such lipoid diseases occur primarily in infants or in the first decades of life. The lipoid deposits are widespread throughout the reticulo-endothelial system, viscera, and bones, and in Gaucher's disease the lipoid stored is a cerebroside, in Niemann-Pick's disease, a phospholipid, and in Hand-Schüller-Christian's disease, cholesterol and cholesterol esters. In Whipple's disease all reported cases have occurred in middle-aged or elderly subjects. The lesions are limited to the small intestine and the associated mesenteric nodes, and the lipid for the most part is neutral fat.

Localized deposits of lipid have been described in the gastric and intestinal mucosa in middle-aged and elderly persons.[21,22] These lesions differ from those of Whipple's disease since they are made up of small groups of cells containing fat droplets and there is no giant cell formation. Clinically, no symptoms have been attributed to these lesions.

Whipple [1] suggested that the fat is abnormal in some way. The evidence of this, in his opinion, was the low saponification number and the presence of the peculiar foreign body giant cell reaction to the fat. He also emphasized the presence of numerous ecchymoses and blood pigment as suggesting some action on capillary walls. In addition, Whipple pointed out that the pathologic changes are limited to the structures concerned with fat absorption while there is no involvement of other lymphatic tissue in the body.

Pearse's [10] patient improved remarkably on the prolonged daily administration of bile salts. Under this regime the symptoms disappeared, vitamin A absorption improved, and the fat content of the stools approached normal. Upon purposeful omission of the medication, the patient had two successive remissions with nausea, indigestion, flatus, and diarrhea as chief symptoms. These symptoms were relieved both times by return to the regime of daily bile salts and the patient was well 1 to 2 years after operation. This remarkable recovery of Pearse's patient on bile salts is hard to explain in view of current theories of fat metab-

olism. Pearse merely suggested that some obscure qualitative factor of bile or bile salts is lacking and that replacement of this unknown factor effects the cure.

Summary

1. The eighth reported case of Whipple's disease, or intestinal lipodystrophy, is described. This case is of particular interest because of the bizarre hematologic picture that caused it to be confused with lymphatic leukemia and hemolytic icterus.

2. Whipple's disease is characterized pathologically by the deposit of lipids in the mucosa of the small intestine and in the mesenteric lymph nodes. Clinically, the disease is marked by asthenia, anemia, arthritis, steatorrhea, abdominal distention and discomfort, and usually progresses to a fatal termination.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

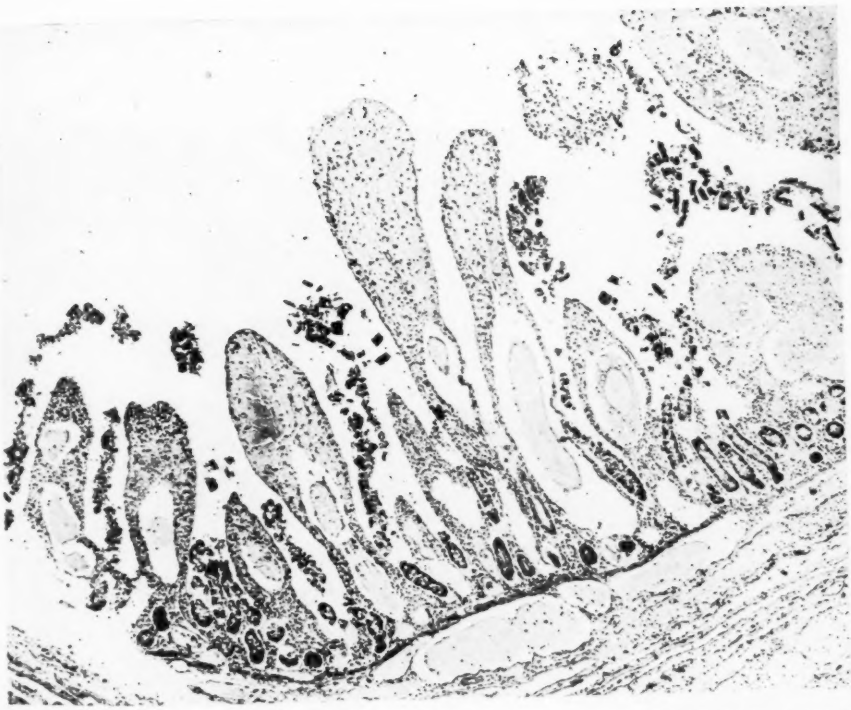
PLATE 188

FIG. 1. Small intestine, showing the mucosa containing the lipoid substance.

FIG. 2. Low-power view of the small intestine illustrating the marked variation and increase in size of the villi due to the lipoid substance. Phloxine-methylene blue stain. × 55.

1

2



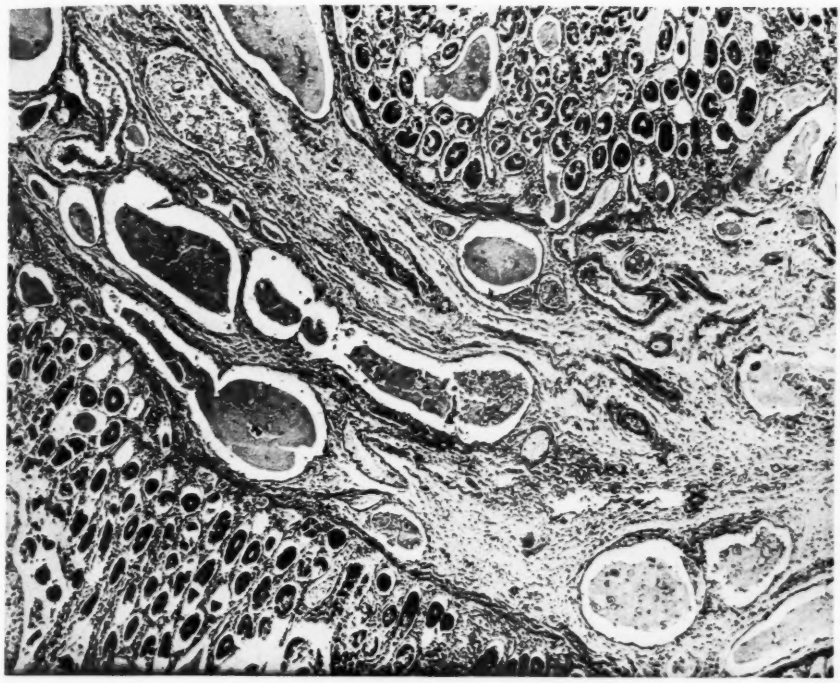
Fitzgerald and Kinney Intestinal Lipodystrophy

PLATE 189

FIG. 3. Low-power view of the small intestine showing the lacteals of the enlarged villi distended by lipoid. Phloxine-methylene blue stain. × 130.

FIG. 4. Section of the small bowel showing mononuclear cells containing the lipoid substance. Phloxine-methylene blue stain. × 260.

3



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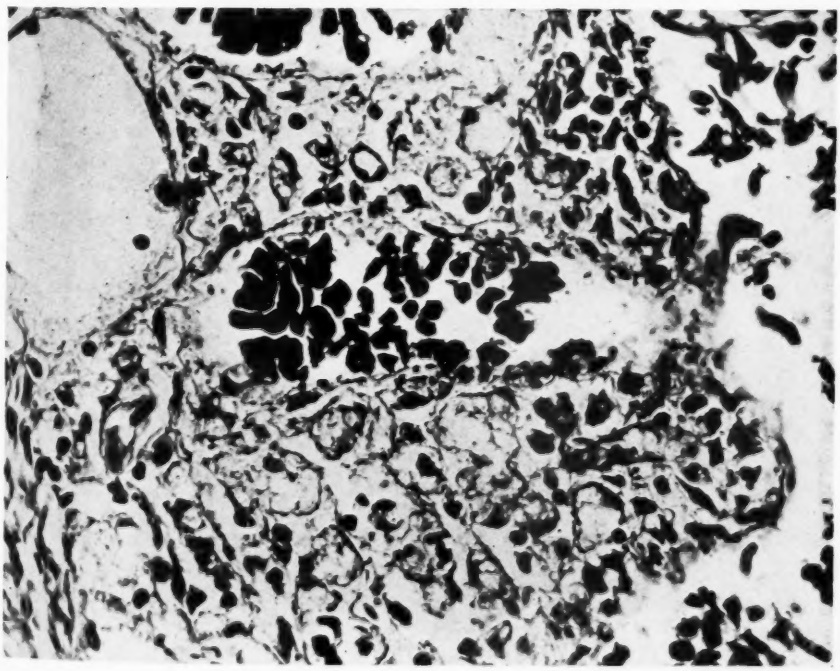


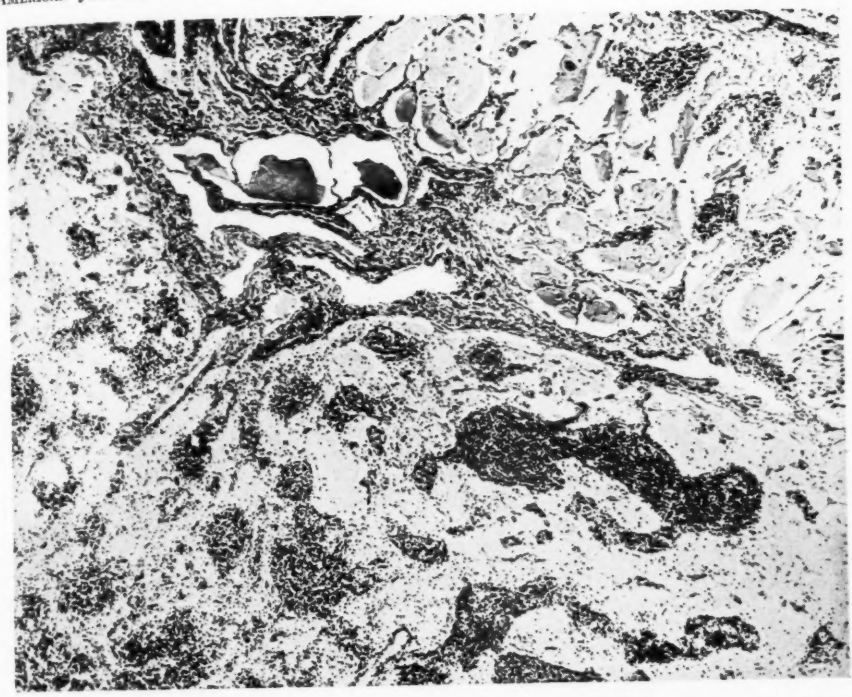
Fitzgerald and Kinney Intestinal Lipodystrophy

PLATE 190

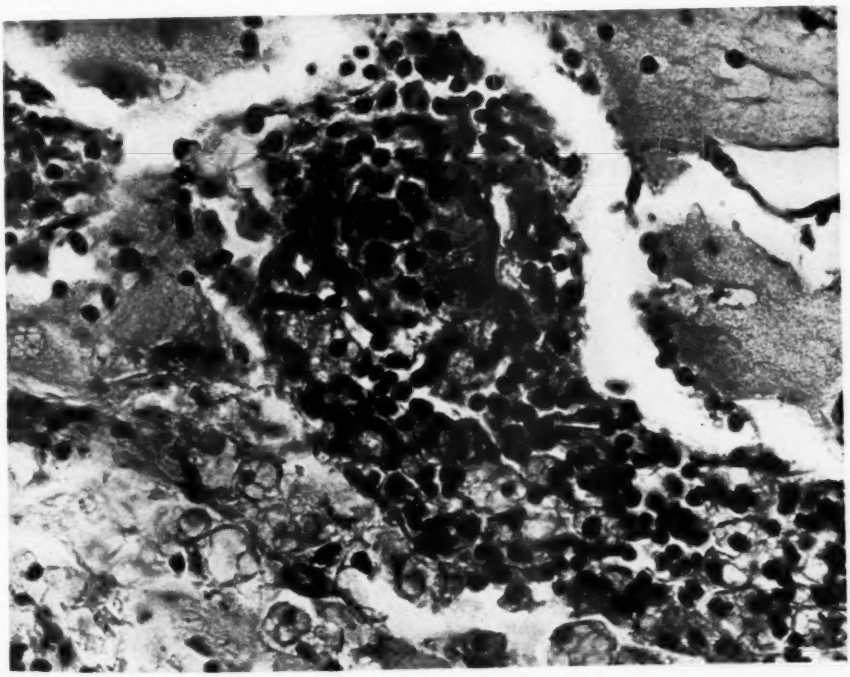
FIG. 5. Low-power view of a mesenteric lymph node showing the distention of the cortical and medullary sinuses. Phloxine-methylene blue stain. × 130.

FIG. 6. Section of a mesenteric lymph node showing the lipoid-laden mononuclear cells. Phloxine-methylene blue stain. × 215.

5



6



Fitzgerald and Kinney Intestinal Lipodystrophy

A PATHOLOGICAL STUDY OF RENAL DAMAGE PRODUCED BY SULFADIAZINE IN RATS

DEVELOPMENT, REPAIR, AND RESIDUA *

K. M. ENDICOTT, M.D., and ARTHUR KORNBERG, M.D.

(From the Pathology Laboratory and the Division of Physiology, National Institute of Health, U. S. Public Health Service, Bethesda 14, Md.)

Since the introduction of sulfonamide drugs as therapeutic agents, there have been numerous reports of renal complications. A fairly complete bibliography of the reported lesions in fatal cases is contained in the paper of Murphy, Kuzma, Polley, and Grill.[1] Studies in various experimental animals have also been described.[2-12] In most of these, renal lesions have been produced by a single dose or a few overwhelming doses of one of the sulfonamides. No extensive studies have been reported concerning the phases of recovery.

The present study was made in connection with a physiological study of the production and prevention of renal lesions. This report will present observations on the morphological aspects of the development, early and late recovery stages, and residual effects of renal damage produced in rats by sulfadiazine. The physiological aspects of the study are presented elsewhere.[13]

METHODS

The development of renal lesions was studied in over 200 albino rats which were placed on diet no. 803 [13] within a week after weaning. This diet consisted of 10 per cent casein, 8 per cent Crisco,† 77 per cent sucrose, and 1 per cent sulfadiazine with appropriate salt and vitamin supplements. At intervals varying from 1 to 30 days after beginning the diet, rats died or were killed and the tissues were fixed in 3.7 per cent aqueous formaldehyde, embedded in paraffin, sectioned, and stained with hemalum-eosin-azure and van Gieson's picrofuchsin.

For the purpose of studying the recovery stages, 12 weanling rats were given diet no. 803 for 30 days. Then, under ether anesthesia, the right kidney was inspected. All 12 rats showed gross evidence of marked renal damage. In 7 rats the right kidney was removed for study. The rats were then given diet no. 819 [13] which differed from diet no. 803 only in that the sulfadiazine was replaced by an equal weight of sucrose. The rats died or were killed after 2 to 15 days and the tissues were examined as before.

For the purpose of studying residual renal lesions, 16 rats were

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† Crisco is a partially hydrogenated vegetable oil made by Procter and Gamble, Cincinnati, Ohio.

given diet no. 803 to produce marked renal damage and the right kidney was then removed for study. After nephrectomy the diet was changed to no. 819 (without sulfadiazine) and the rats were killed 3 months later and studied as before.

Observations

The earliest lesions were in the renal papillae and consisted of a chalky striation. The surface of the kidney appeared normal. In rats killed after longer intervals, the chalky striation extended into the cortex, and in most rats surviving 30 days the striation reached from the tip of the papilla to the capsule, following the course of the collecting tubules. The kidneys in these rats were enlarged and pale tan, and were covered with a fine, chalky white stippling.

Inspection of the stained sections showed a striking dilatation of the tubules, visible without magnification (Figs. 1 to 5). This dilatation was more or less restricted to that portion of the kidney which showed the chalky striation grossly. In sections prepared by fixing the kidneys in formaldehyde vapor,[14] cutting frozen sections, transferring them directly to dry slides, and mounting in xylol-clarite, many tubules were filled with crystals and amorphous débris. Most of the crystals were identified by their characteristic shape as acetylsulfadiazine. In ordinary paraffin sections, the crystals were not seen.

The tubular dilatation was noted first in the papilla and later extended to involve the distal convoluted tubules and ascending limb of Henle's loop. In a few kidneys the dilatation involved the entire nephron, including the glomerular capsule. Many of the dilated tubules showed only a flattening of the epithelium. In others, the epithelium was basophilic and showed an increase in number of nuclei or nuclear pyknosis. Epithelial necrosis was encountered very rarely. The epithelium of the dilated tubules rarely contained fat, but basal, fine, fat droplets were demonstrated occasionally by fat stains of frozen sections in the nondilated proximal convoluted tubules and Henle's loop. Epithelial calcification was rare.

Dilated tubules often contained oxyphilic casts. Many of these were coated with neutrophilic leukocytes. In such casts, fan-shaped, empty spaces representing the spaces once occupied by sulfonamide crystals were often present. In some rats no lesions other than tubular dilatation were noted.

The interstitial reaction varied considerably. In the early stages there was occasionally an infiltration of neutrophils around some of the dilated tubules. Intertubular edema was more common. Later an in-

crease of fibroblasts occurred. Occasionally such fibroblasts were arranged concentrically about a dilated and pus-filled collecting tubule producing a picture suggestive of a small granuloma. In some rats surviving 1 month, slight to moderate fibrosis was seen, chiefly in the form of collars about collecting tubules, but also to a lesser extent as a delicate, diffuse, medullary fibrosis extending occasionally into the cortex.

Lesions of the renal pelvis and ureters occurred rather infrequently and were most common in rats which showed few or no intrarenal lesions. A few minute calcareous concretions or oxyphilic casts containing fan-shaped spaces were noted in a few rats. Several rats showed hydronephrosis attributed to impaction of such débris in the ureters. A few rats showed hyperplasia and squamous metaplasia of the renal pelvic epithelium, but in most rats the renal pelvis and ureters were histologically negative. The urinary bladder was not examined regularly.

In spite of diligent search, lesions of renal blood vessels or glomerular tufts were not encountered.

Repair. In the kidney of one rat killed 2 days after discontinuing sulfadiazine administration there was no gross or microscopical evidence of recovery. In rats killed after 5 days there was definite gross and microscopical evidence of beginning renal recovery which was more complete the longer the animal lived. The gross enlargement and chalky stippling decreased and the kidneys became darker than at the time of operation. The cortical surface developed a fine pitting. Sulfonamide crystals disappeared from the tubules. Histologically, the tubular lumina became free of pus and casts, and the epithelium showed heaping up of nuclei, rather numerous mitoses, and cytoplasmic basophilia. Dilatation decreased and many tubules had a normal appearance. The rate of recovery was variable. One rat had recovered more in 5 days than another had in 11 days. However, in all rats some evidence of damage persisted. Nearly all kidneys showed a slight to moderate fibrosis in the medulla and most showed a partial destruction of a number of nephrons.

This destruction of nephrons was most apparent in the subcapsular area where distal convoluted tubules had collapsed and atrophied. This resulted in a decrease of thickness of the supraglomerular zone so that glomeruli came to lie against the capsule. In areas of tubular destruction, a slight fibrosis and lymphocytic infiltration was observed occasionally.

Residual Lesions. All 18 rats surviving 3 months after cessation of sulfadiazine administration showed residual renal damage. Grossly,

the kidneys were normal in color but showed a nodular surface with many small retracted scars and an adherent capsule. They cut with increased resistance.

Microscopically, there was focal to diffuse absence of the supraglomerular zone of convoluted tubules. In these areas of cortical atrophy, numerous shrunken but otherwise normal glomeruli lay side by side without the normal complement of intervening tubules. Such areas showed fibrosis, lymphocytic infiltration, and scattered remnants of tubules. Often, at least one-half of the glomeruli visible in the sections were located in such scars. Usually a broad band of fibrous tissue extended from such cortical scars into the medulla. In the band of scar tissue there were normal and atrophic collecting tubules. The inner cortex and medulla showed moderate to marked focal to diffuse fibrosis with an apparent decrease in the number of collecting tubules. No definite vascular lesions were noted. In the portions of the cortex not involved in atrophy and scarring there was hypertrophy of glomeruli and tubules.

Discussion

The mechanism of the renal damage produced by sulfonamides is unknown. Most authors attribute the tubular lesions to mechanical irritation of the epithelium by the sharp crystals of sulfonamide precipitated there. Some authors, however, suggest that the sulfonamides exert a direct toxic effect on the epithelium of the tubules. In the present study there is some evidence which supports the latter hypothesis. Some kidneys showed massive deposition of sulfonamide crystals in the tubules with no epithelial reaction while other kidneys showed relatively slight deposition of crystals with marked epithelial degeneration and proliferation.

The absence of glomerular and other renal vascular lesions in this series of over 200 rats with marked renal damage indicates that, in the rat, these structures are not particularly susceptible to injury by sulfadiazine. The extreme infrequency of epithelial calcification in this series is also of interest in view of the fact that it is reported to have occurred regularly under different experimental conditions in other laboratories.

The renal lesions encountered in the present study and those reported by others in rats, dogs, monkeys, mice, and man are all quite similar. The collecting and distal convoluted tubules appear to be the chief sites of damage. In the rat, we have shown that permanent renal damage can result from sulfadiazine administration. It is not unlikely that similar residual lesions might be found in other experimental animals and in man if a search were made.

Summary

The renal lesions which develop in rats given a low casein diet containing 1 per cent sulfadiazine [13] have been studied histologically. These lesions appear first in the renal papillae and extend later into the cortex. There is a deposition of sulfonamide-protein casts in the tubules accompanied by dilatation of the tubules, degeneration and proliferation of tubular epithelium, and leukocytic exudation. Interstitial edema, leukocytic infiltration, and fibrosis are common. Grossly, the kidneys are enlarged, pale, and stippled with chalky white markings. The cut surface shows chalky radial striations extending out from the papilla.

Withdrawal of the drug after the development of marked renal lesions results in partial recovery. Some damaged tubules recover completely while others undergo atrophy and disappear. Kidneys examined 100 days later are nodular and scarred, and show destruction of the distal convoluted tubules of as many as one-half of the nephrons. There is prominent fibrosis.

The possible occurrence of similar permanent renal damage in other experimental animals and in man is suggested.

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DESCRIPTION OF PLATES

PLATE 191

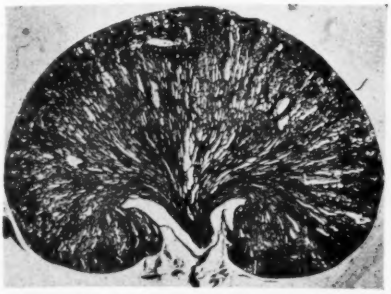
FIG. 1. Marked dilatation of renal tubules. Hematoxylin-eosin-azure stain. × 4.

FIG. 2. Normal kidney for comparison. × 4.

FIG. 3. Collecting tubules showing cellular and protein-sulfonamide casts. Spaces are left in cast after sulfonamide is dissolved. × 170.

FIG. 4. Collecting tubule containing pus and showing epithelial degeneration. There is also an interstitial leukocytic infiltration. × 340.

FIG. 5. Collecting tubules showing increased number of nuclei. × 340.



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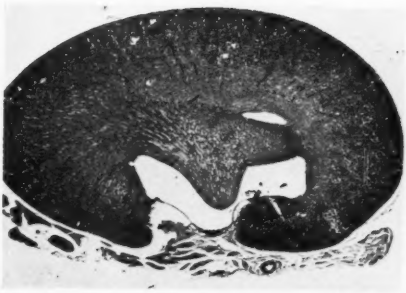

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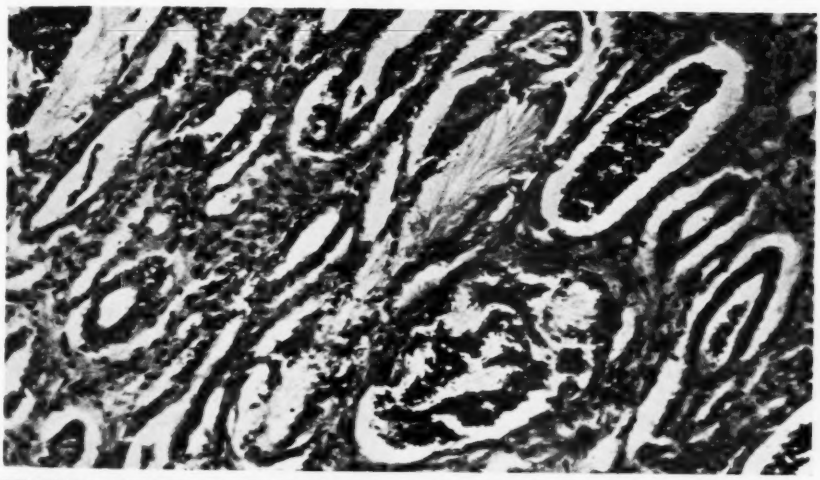

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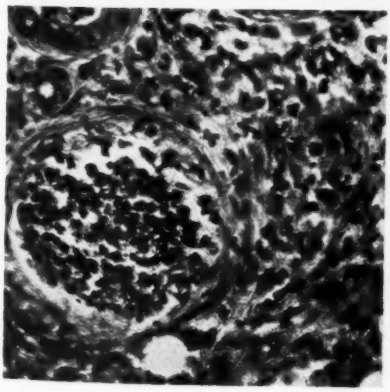

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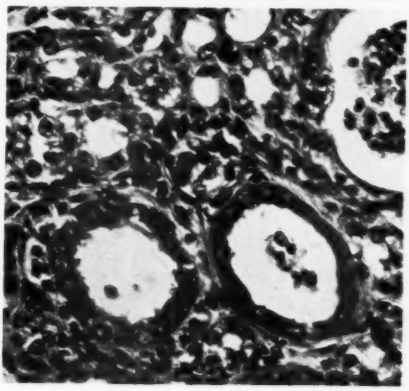

5

Endicott and Kornberg

Renal Damage Produced by Sulfadiazine

PLATE 192

FIG. 6. From left to right: normal kidney, enlarged stippled kidney after 30 days of sulfadiazine administration, and nodular scarred kidney 3 months after cessation of sulfadiazine administration. × 1.

FIG. 7. Cortical scar from a nodular kidney, showing lymphocytic infiltration, agglomeration of glomeruli, and atrophy of tubules. Van Gieson stain. × 250.

FIG. 8. Cortical scar. × 250.

FIG. 9. Atrophy and collapse of supraglomerular tubules. × 250.

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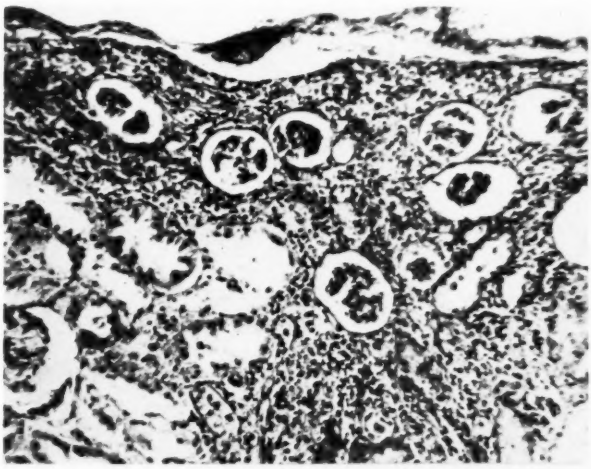

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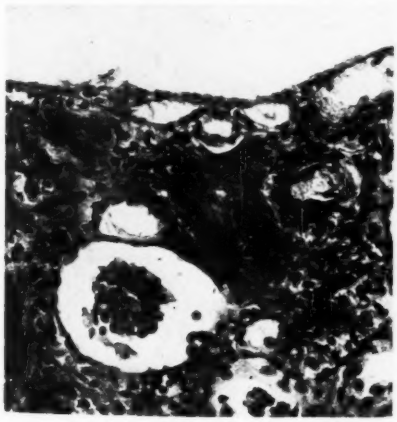

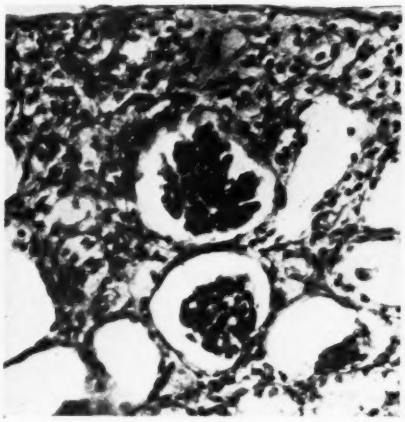

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Endicott and Kornberg

Renal Damage Produced by Sulfadiazine

STUDIES IN VITRO ON THE PHYSIOLOGY OF CELLS

Histologic Reactions of Living Tissues to Hypotonic Solutions*

Robert Schrek, Major, M.C., A.U.S.
(From the Tumor Research Unit, Veterans Administration, Hines, Ill.)

A previous study [1] demonstrated, by the method of unstained cell counts, that cells differed in their reaction to distilled water. Testicular cells in suspension were found to be quite sensitive to the lethal and lytic action of this hypotonic fluid. Thymic cells were not as susceptible and suspensions of these cells developed a gelatinous precipitate. No precipitate, however, was observed in suspensions derived from the spleen.

The studies on the effect of distilled water have been continued by exposing freshly excised viable tissues to hypotonic and isotonic solutions and then preparing histologic sections of the tissue. By means of this deferred histologic method, it was possible to demonstrate marked differences in the physiologic reaction of cells to distilled water.

The objectives of the present work were: (1) to check the previous findings [1] on the effect of hypotonic solutions on cells in suspension; (2) to investigate the utility of the deferred histologic method as a means of studying the physiology of excised tissues; and (3) to obtain additional data on the reactions of tissues to distilled water and to Ringer's and isotonic sucrose solutions.

Methods

Thymus, testis, and other organs of the rat and rabbit were cut with a sharp knife into pieces measuring 2 to 5 mm. in length. Each piece of tissue was placed in a 100 by 13 mm. test tube which contained 5 cc. of fluid and which was stoppered with a black rubber stopper. The tubes were placed in a perforated metal box and rotated at 80 r.p.m. in a water bath maintained at 2°, 38°, or 45°C. After incubation, the gross appearance of the tissue and of the fluid was noted. The tissue was then fixed in Bouin's or Zenker's solution and paraffin sections were prepared.

The fluids used in these experiments were distilled water, Ringer's solution, and 0.33 molar sucrose solutions, to each of which was added 5 per cent of a phosphate buffer ($\frac{1}{15}$ molar) having a pH of 7.6.

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Thymus

To study the initial reactions, thymic tissue was exposed to phosphate-water at approximately 2°C. for 30 minutes and then fixed, cut, and stained. In the peripheral zone of the section, the small cortical cells were separated by clear spaces evidently due to interstitial edema (Fig. 1). The cells were round or oval and were normal in size or slightly enlarged. The nuclei stained intensely and some of them had the shape of a horseshoe or of a ring which surrounded a small amount of central cytoplasm. Reticular cells close to the periphery were considerably enlarged (Fig. 2), with well defined cell walls and widely spaced, fine granules in the cytoplasm. The lightly staining nuclei of these cells were also enlarged and had conspicuous nuclear membranes and prominent nucleoli. The histologic changes suggested that these reticular cells had been killed by the distilled water and were undergoing lysis.

In a few areas near the periphery of the section, the cellular structure of the treated thymus was partly replaced by sheaths of thin, basophilic fibrils which formed whorl-like loops around small acinus-like structures (Fig. 3). The ends of the loops were frayed and pointed towards the periphery of the section. The sheaths of fibrils were somewhat loose, and intermixed with the fibrils were round and spindle-shaped thymic cells in moderate numbers. The nuclei of some of these cells appeared elongated.

The acinus-like structure at the center of the whorl of fibrils had, usually, a small, clear space surrounded by two to four flat or cuboidal cells (Fig. 3). The nuclei were moderate in size and stained deeply and uniformly. The cytoplasm was abundant and stained deeply with eosin. These structures were probably Hassall's corpuscles.

More prolonged incubation of thymic tissue with phosphate-water (3 hours at 2°C.) resulted in a marked increase in the number of basophilic fibrils (Fig. 5). The sheaths of fibrils were numerous, thick, and dense, and did not take the silver stain for reticulum. The sheaths formed whorls around small acinus-like structures. The cells in these structures were not so well defined as in the previous section but many of the cells still had deeply staining cytoplasm and nuclei. Small thymic cells were sparse in areas having many fibrils but were numerous in other parts of the cortex (Fig. 4). The nuclei of the cortical cells were horseshoe or ring-shaped. The reticular cells were few in number, apparently due to the lysis of most of them. The persisting markedly enlarged reticular cells had large or small nuclei which stained lightly and homogeneously (Fig. 4). A few large, clear spaces were present in the center of the section and probably represented

areas which were originally occupied by reticular cells (Fig. 5). It is to be noted that the small cortical cells and the large reticular cells reacted differently to the phosphate-water.

The early reactions of thymic tissue to hypotonic solutions at 38°C. were studied by exposing the tissue to a mixture of 7 parts of phosphate-water and 3 parts of phosphate-Ringer's solution for 30 minutes. Sections of the tissue (Fig. 6) had a central area composed of fairly normal cells. In the peripheral zone, the cortical cells were normal in size. A few nuclei were horseshoe-shaped as observed in tissue treated with water at 2°C. Most of the nuclei, however, were round or oval and lightly stained. It is probable that these cells were dead at the time of fixation and that the observed changes in the nucleus were in part due to autolysis at 38°C. In this section, no fibrils were seen. The reticular cells in the peripheral zone were markedly enlarged with very large, poorly staining nuclei.

Thymic tissue exposed to phosphate-water at 38°C. for 1 hour had a moderate number of basophilic fibrils (Fig. 7). Some of the fibrils were isolated from each other; others formed sheaths or a loose network. The sheaths of fibrils did not form whorls or loops. In some sections, acellular areas had fine basophilic granules.

Changes were observed in the blood vessels of the thymic tissue treated with distilled water. In early stages some of the blood vessels had a small amount of amorphous débris which represented the remnants of lysed red blood cells. The endothelial cells of capillaries and small arterioles were enlarged and extended into the lumen (Fig. 17). The cell wall was prominent; the cytoplasm was unstained; the nucleus was large and round, had a light bluish, muddy color, and had no definite granules. The smooth muscle cells of the muscularis were not enlarged; the cytoplasm stained intensely; the nuclei were moderately enlarged and also stained deeply.

In one section of thymus exposed to a mixture of phosphate-water (9 parts) and phosphate-Ringer solution (1 part) at 38°C. for 30 minutes, a small vein was cut diagonally (Fig. 8). The nuclei of the endothelial cells were moderately enlarged. There was a layer of subendothelial cells which were markedly enlarged, with edematous unstained cytoplasm and centrally placed, enlarged, round, poorly stained nuclei. These enlarged cells were probably smooth muscle cells.

Thymic tissue exposed for 1 hour to Ringer's or sucrose solution at 2° and at 38°C. showed none of the changes described above and appeared normal. After prolonged incubation (4 hours at 45°C.) the tissue had relatively few cortical and medullary cells (Fig. 9). In some cases, the sections were almost acellular but the fibrous framework

and the blood vessels of the tissue remained intact. It should be noted that, in these experiments, the solution in which the thymic tissue was incubated became turbid after incubation. Smears and sections of the turbid fluid showed intact, well preserved cells. It would seem, then, that the thymic cells were gradually washed out from the tissue on prolonged incubation with Ringer's or sucrose solution.

It may be concluded that incubation of thymic tissue with distilled water caused nuclear changes in the cortical cells, swelling and lysis of reticular cells, production of a basophilic granular and fibrillar precipitate within the tissue, and cytoplasmic and nuclear enlargement of the endothelial cells of blood vessels.

Spleen and Lymph Node

Splenic tissue of the rabbit was exposed to phosphate-water at 2°C. for 6 hours. The capsule was normal but the cells beneath the capsule showed vacuolization of the cytoplasm and enlargement and poor staining of the nuclei. The cut edge of a rat spleen which was exposed to the phosphate-water for 6 hours had a small amount of fibrillar precipitate (Fig. 10). The thin fibrils were usually eosinophilic but in a few areas they were basophilic. In other experiments, no fibrils were seen in the sections. The splenic pulp and the malpighian bodies in the center of the section were fairly normal except for lysis of the red blood cells.

In several experiments, splenic tissue was exposed to phosphate-water at 38°C. The only changes observed in the section were swelling and vacuolization of the cytoplasm of the cells beneath the capsule.

To recapitulate, the histologic changes of splenic tissue treated with phosphate-water consisted chiefly of the enlargement and vacuolization of the subcapsular cells. Occasionally there was also the formation of a small amount of eosinophilic and basophilic precipitate. The changes were not constant in different experiments and were never as striking as those observed in thymic tissue.

A human cervical lymph node was exposed to phosphate-water for 30 minutes at 38°C. (Fig. 11). The architecture was fairly well preserved. Nearly all of the cells in the node, particularly those near the periphery of the section, were moderately or markedly enlarged, with vacuolization of the cytoplasm. Many of the nuclei of the peripheral cells were enlarged and stained poorly. Control tissues exposed to phosphate-Ringer's and phosphate-sucrose solutions at 38°C. for 30 minutes were almost identical in histologic appearance to tissue fixed immediately after excision.

A cervical lymph node of a rabbit was exposed to buffered water at 2°C. for 5 hours. On examination, the sections showed enlargement

of the lymphocytes with clear, unstained cytoplasm and small, deeply staining nuclei (Fig. 12). In addition, a few small, superficial areas had basophilic fibrils forming a loose network. In some experiments exposure of lymph nodes to phosphate-water failed to cause enlargement of the lymphocytes or the formation of fibrils. For example, a small para-aortic node exposed to phosphate-water at 38°C. for 30 minutes was, on histologic section, fairly normal in appearance (Fig. 13). In another lymph node from the cervical region of a rabbit, the only significant change was an intercellular, homogeneous, eosinophilic precipitate in the medulla.

It is evident that lymph nodes varied in their reaction to phosphate-water. The cause for the variation was not definitely established. The small para-aortic node of the rabbit was normal in control sections and was not affected by incubation with phosphate-water. On the other hand, the human cervical lymph node was large and firm and showed considerable inflammatory and hyperplastic changes in control sections. Exposure of this node to phosphate-water caused marked swelling of the cytoplasm of the lymphocytes. The reactivity of the nodes to hypotonic solutions may depend on the physiologic activity of the tissue in the animal. The number of lymph nodes studied, however, was not sufficient to establish definitely whether inflammatory processes in the node increase the reactivity of the tissues to hypotonic solutions.

Testis

The earliest effect of phosphate-water on the testis of a rabbit was observed when the tissue was incubated at 38°C. for ½ hour in a fluid consisting of 3 parts of phosphate-water and 7 parts of phosphate-Ringer's solution. The sections of the tissue showed an almost complete disappearance of mitotic figures. The nuclei of most of the spermatogenic cells were large and showed a few large chromatin granules.

Incubation with a more hypotonic solution (9 parts of phosphate-water and 1 part of phosphate-Ringer's) caused the testicular cells to become swollen until they completely filled the seminiferous tubules (Fig. 14). The cytoplasm was vacuolated and the nuclei stained faintly but maintained their structural characteristic of coarse chromatin granules. A few nuclei were very large and consisted of fine, lightly stained, basophilic granules. These giant nuclei did not have definite nuclear membranes. In contrast to the spermatogenic cells, the interstitial cells were well preserved, appeared normal in size, and had deeply staining nuclei and cytoplasm. In many experiments, however, no interstitial cells were seen in the sections; they may have been washed out from the tissue by the distilled water.

Exposure of rabbit testis to phosphate-water at 38°C. for 30 minutes caused almost complete destruction of the spermatogenic cells (Fig. 15). The tubules were filled with amorphous, eosinophilic débris. Some of the tubules, however, still had many well preserved, darkly staining, elongated nuclei of spermatocytes.

When the testis of the rat was exposed to hypotonic solutions, the spermatogenic cells were also destroyed with the production of eosinophilic detritus. There was a tendency, however, for the phosphate-water to remove or dissolve the débris, leaving the fibrous framework which usually became distorted and collapsed. The most satisfactory histologic picture was obtained in a rat testis incubated without shaking at 4°C. for 6 hours. The section consisted of thin fibrous strands which enclosed large, irregular-shaped, clear spaces (Fig. 16). In the persisting fibrous trabeculae there were numerous small blood vessels. The endothelial cells were enlarged and had clear cytoplasm and large, round, pale nuclei (Fig. 17). Many of the small arterioles had no endothelial cells, evidently as a result of their lysis by the phosphate-water. Capillaries were not seen in this section, but in testicular tissues exposed for a shorter time the capillaries were found to consist of swollen endothelial cells.

Testis exposed to buffered Ringer's solution at 38°C. for 1 hour showed a separation of the tubules, presumably due to intertubular edema (Fig. 18). Mitotic figures were numerous, although somewhat reduced in number as compared to the control, untreated tissue. The architectural arrangement of the cells in the tubules was well preserved. The cells and their nuclei were normal in size and in stainability.

Testis exposed to phosphate-sucrose solution at 2°C. for 3 hours showed, on microscopic section, a marked reduction in the number of mitotic figures. Many tubules had large giant cells with two to ten nuclei of moderate size, which were either vesicular or stained deeply and uniformly (Fig. 19). An occasional cell had a deeply staining, giant-sized nucleus. Multinucleated cells were also observed in testis exposed for a short time to a hypotonic solution and then to a phosphate-Ringer's solution.

Salivary Gland and Other Tissues

The submaxillary gland of the rabbit was exposed to phosphate-water at 38°C. for 1 hour. The sections had normal architecture (Fig. 21). There was no enlargement of the epithelial cells, not even of the peripherally placed ones which were in direct contact with the hypotonic solution. In contrast, the numerous arterioles throughout the tissue showed lysis of red blood cells and swelling of the endothelial cells. The nuclei of the cells of the tubules and acini were small, some-

what irregular in shape, and stained deeply and uniformly. These nuclei were apparently pyknotic. The cytoplasm of the tubules stained more deeply than in control sections. The observed changes in the nuclei and cytoplasm probably indicate that the epithelial cells were dead at the time of fixation.

The cells of the glandular tissue exposed to phosphate-Ringer's and sucrose solutions had, for the most part, nuclei which were fairly normal in microscopic appearance. A few nuclei, however, were small and stained deeply and homogeneously.

In sections of liver exposed to distilled water at 2°C. for 7 hours the peripheral zone consisted of enlarged cells with vacuolated cytoplasm and normal or small, darkly staining nuclei. The enlargement of the peripheral hepatic cells obliterated the sinuses. In the central part of the section, the architecture and the cellular detail were normal. In other experiments (liver exposed to 38°C. for 1 hour), the sinuses throughout the section were filled with deeply staining, homogeneous eosinophilic material.

Transplantable Tumors

A strain of leukemic myeloblastoma was grown as subcutaneous nodules in dba mice. The tumor was excised and exposed to phosphate-water at 38°C. for 30 minutes. Sections of the treated tissue (Fig. 22) showed a peripheral zone composed of eosinophilic débris and a few degenerating cells. Beneath the layer of detritus was a thin zone in which the cells were enlarged, with clear or lightly stained cytoplasm and large, muddy, lightly stained nuclei. In the center of the section the cells were well preserved and the nuclei well stained. Mitotic figures, however, were few in number although they were numerous in control sections.

Other transplantable tumors, sarcoma 180 and melanoma of mice, and R39 of rats, were exposed to phosphate-water at 38°C. for 1 hour and their behavior was found to be similar. Sections showed enlargement of the malignant cells, the cytoplasm was unstained, and the nuclei of most of the cells were apparently normal. A few nuclei near the periphery of the section were slightly enlarged and had a muddy stain. There were no mitotic figures.

Discussion

A Histologic Method of Studying the Physiology of Excised Tissues

The purpose of the customary histologic and pathologic methods is the determination of the morphologic appearance of the cells and tissues in the normal, diseased, or experimental animal. To accomplish this objective, the tissues are fixed, *i.e.*, killed, immediately after their

removal from the animal's body. The present series of experiments on cellular physiology has as its objective the determination of the reactions of living cells *in vitro* to reagents. It is well known that freshly excised tissues are composed of living cells. Any histologic changes occurring in the tissue after excision are the result of the reaction of the living, dying, or dead cells to the particular environment. This line of reasoning led to the utilization of the deferred histologic method in which the excised tissue is first exposed to the reagent, then fixed, and studied histologically.

A review of the literature shows that several investigators have used the deferred histologic method to study specific problems. Workers who use Warburg's manometric method [2] frequently examine tissue histologically after studying its metabolism. Usually these histologic studies are performed as a check on the type and morphologic integrity of the tissue. Sometimes the tissues were studied to determine the changes induced by the artificial environment. Okamoto,[3] for example, found that rat tumors subjected to anaerobiosis maintained their morphologic structure for 3 days at 37°C., whereas hepatic tissue under identical conditions developed degenerative changes. The deferred histologic method was used also by Colwell [4] who studied autolysis in excised hepatic tissue subjected to irradiation.

The deferred histologic method has a few limitations. In the first place, the method does not differentiate between living and dead cells. It should be emphasized that marked histologic changes do not necessarily mean that the cell is dead. On the other hand, certain chemicals (fixatives, for example) may kill the cell without producing any perceptible histologic change. A second limitation of the method is that the reagent is not in direct contact with all cells. The reactions, then, depend in part on the diffusibility of the reagent through the tissue. In many of the present experiments only the peripheral zone was affected. It is therefore important not to trim the tissue used for histologic section. A third limitation is the difficulty in obtaining quantitative data with this method inasmuch as histologic observations are usually not suitable for quantitative analysis. In spite of these limitations, the deferred histologic method has a distinct place in the armamentarium of the cellular physiologist.

The Effect of Hypotonic Solutions on Cells and Tissues

Cells differed markedly in their reaction to hypotonic solutions. At least three types of reactions were observed. One type was shown by the spermatogenic cells of the testis, the reticular cells of the thymus, and the endothelial cells of the blood vessels. On incubation

with phosphate-water, these cells became swollen, the cytoplasm stained lightly or was unstained, and the nuclei were large and poorly stained. Ultimately these cells were lysed, leaving a clear space or a small amount of eosinophilic débris.

The cells of the salivary gland exemplified a second type of reaction to hypotonic solutions. Exposure to phosphate-water did not enlarge the parenchymal cells or their nuclei and did not decrease the staining intensity of the cells. In fact, the nuclei stained more intensely after incubation of the living tissue with distilled water. This second type of reaction was observed in some experiments with cardiac muscle, the smooth muscle cells of blood vessels, and the stratified epithelium of the tongue.

A third type of reaction to hypotonic solutions was characterized by swelling of the cell, but the nucleus failed to enlarge. The cytoplasm stained poorly while the staining intensity of the nucleus was normal or greater than normal. Liver cells in contact with phosphate-water showed this type of reaction.

Some cells varied in their reaction to hypotonic solutions. In some experiments, the lymphocyte of the lymph node showed little or no histologic change. In other experiments, apparently under the same conditions, the lymphocyte became enlarged, the cytoplasm was voluminous and unstained, but the nucleus remained normal in size and staining intensity. A detailed review of the experimental and control sections was made to determine the reason for the variation in the reactions of the lymphocyte. It seemed that the resting lymphocyte of a normal lymph node did not become enlarged on treatment with distilled water but the active lymphocyte of inflammatory nodes apparently reacted strongly to the hypotonic solutions. These findings suggested that the reactivity of the lymphocyte to distilled water depended on the physiologic state of the cell.

The histologic changes that were observed in cells treated by hypotonic solutions are evidently the reflection of physiologic reactions within the cell. The enlargement of the spermatogenic, reticular, and endothelial cells in hypotonic solutions and the poor staining of the cytoplasm are evidently the result of the excessive penetration of water into the cell. The enlargement and the light staining of the nuclei of these cells may be assumed to be due to the entrance of water into the nuclei. Evidently, the cell wall and the nuclear membrane of these cells are permeable to water. On the other hand, certain cells, such as the parenchymal cells of the salivary gland, failed to enlarge when treated with phosphate water. This finding may be interpreted in two ways: Either the cell wall is not readily permeable to water or it is

so rigid that it does not permit the enlargement of the cell. The enlargement of some types of cells (hepatic cells and lymphocytes of inflammatory lymph nodes) without an associated enlargement of the nuclei may be interpreted as indicative of permeability of the cell wall to water but an impermeability or a rigidity of the nuclear membrane. It would seem, then, that the observed differences in the reactions of cells to hypotonic solutions are probably due to differences in the characteristics of the cell wall and the nuclear membrane.

The histologic changes within the cells treated with hypotonic solutions were associated with the formation of extracellular precipitates. Different types of precipitates were observed. In two isolated experiments a small amount of precipitate that stained homogeneously with eosin developed in the medulla of a lymph node and in the sinuses of hepatic tissue treated with phosphate-water. The precipitates were not definitely associated with the lysis of cells and they may have resulted from the exudation of a protein from the intact cells.

A heavy, amorphous, eosinophilic precipitate was formed as a result of the lysis of the spermatogenic cells of the testis of the rabbit. In contrast, no definite precipitate followed the lysis of the reticular cells of the thymus and the endothelial cells of the blood vessels. It seems that the products released by the lysis of the reticular and the endothelial cells were washed away or dissolved in the supernatant fluid, but the products of lysis of the testicular cells of the rabbit were less soluble.

In some of the experiments with lymph node or spleen there was formed a granular or fibrillar precipitate which was eosinophilic or slightly basophilic. The precipitate was presumably the result of the lysis or the rupture of lymphocytes. The inconstancy of the formation of the precipitate in different experiments may be due to a tendency of the supernatant fluid to remove the precipitate from the tissue.

Exposure of thymic tissue to hypotonic solutions resulted in the formation of a characteristic precipitate which was usually fibrillar in structure and was basophilic in staining. The formation of this precipitate in thymic tissue is evidently correlated with the gelatinous precipitate which was previously observed on the addition of distilled water to suspensions of thymic cells.[1] The histologic sections indicated that the precipitate was derived from the small cortical cells of the thymus. It is possible that Hassall's corpuscles played a rôle in the formation of the basophilic, fibrillar precipitate.

Summary

Freshly excised tissue was exposed to distilled water at 2°, 38°, and 45°C. and was then fixed, sectioned, and stained.

Under these conditions the testis showed disappearance of mitotic figures, then swelling and ultimately lysis of the spermatogenic cells. The fibrous framework, however, was well preserved.

In the thymus, the reticular cells underwent swelling and lysis. The cortical cells developed nuclear changes and were finally replaced by a fibrillar or granular, basophilic precipitate.

The reaction of the spleen and lymph node to hypotonic solutions varied and was usually slight. In some cases, however, the lymphocytes became enlarged and occasionally a small amount of granular or fibrillar precipitate developed.

Exposure to distilled water did not affect the acini or ducts of the salivary gland but did cause pyknosis of the nuclei.

Blood vessels showed enlargement and finally lysis of the endothelial cells and little or no change was seen in the muscularis.

The cells of a few transplantable tumors became large and edematous and, in some cases, showed degenerative changes.

Incubation of the testis with 0.33 molar sucrose solution resulted in the formation of large multinucleated giant cells in the tubules.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 193

FIGS. 1, 2, and 3. Thymus (rat) exposed to phosphate-water (5 per cent phosphate buffer, pH 7.6, in distilled water) for 30 minutes at 2°C. × 700.

FIG. 1. The small cortical cells of the thymus are separated from each other by a small amount of intercellular edema. The cells are normal in size or slightly enlarged. The nuclei stain deeply and have the shape of a horseshoe or ring.

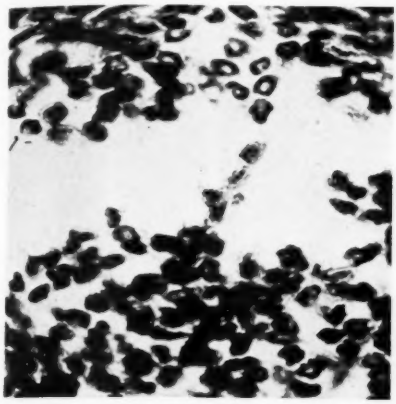
FIG. 2. The photomicrograph is taken from an area composed of a mixture of cortical and reticular cells. The cortical cells are small and have small, deeply staining nuclei. The reticular cells are enlarged, the cytoplasm is unstained, and the nuclei are considerably enlarged and stain lightly. A few nuclei have prominent nucleoli.

FIG. 3. The small acinus-like structure is composed of two flattened cells with deeply staining cytoplasm and nuclei. The cells surround a small, clear space. A few basophilic fibrils loop around the acinus-like structure.

FIGS. 4 and 5. Thymus (rat) exposed to phosphate-water for 3 hours at 2°C. × 350.

FIG. 4. The cortical cells are normal in size and have small, dark, nuclei. The reticular cells are markedly enlarged and have unstained cytoplasm; the nuclei stain very faintly. The nuclei of some reticular cells are not seen in this section.

FIG. 5. The cortical cells have been largely replaced by several bundles of fine and coarse, deeply staining fibrils which loop around a poorly preserved acinus-like structure. The reticular cells in the medulla of the thymus have completely disappeared leaving large, clear spaces.



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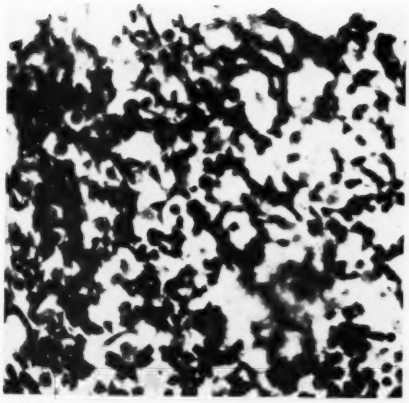

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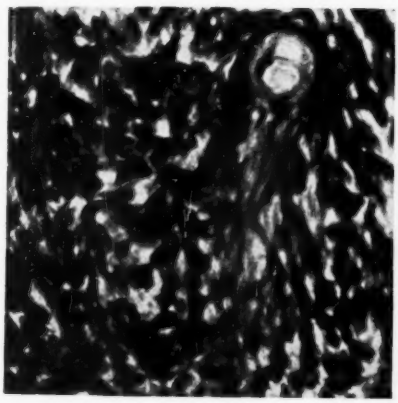

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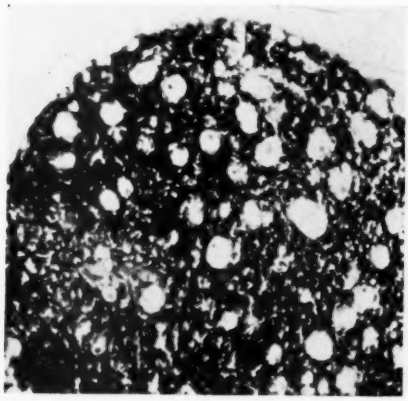

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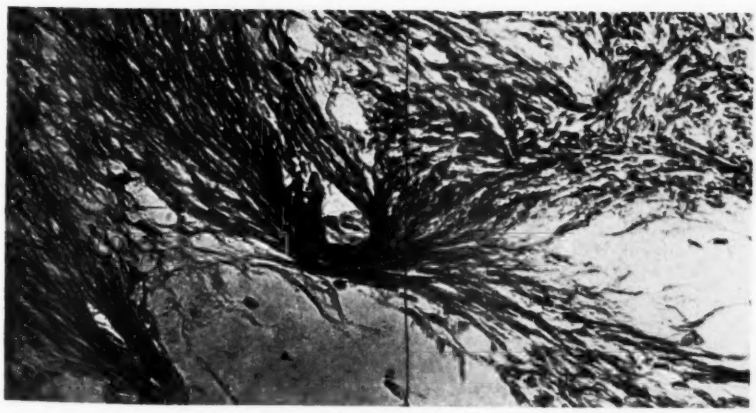

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PLATE 194

FIG. 6. Thymus (rabbit) exposed for 30 minutes at 38°C. to a mixture of 7 parts of phosphate-water and 3 parts of phosphate-Ringer's solution. × 170. The cells in the peripheral zone are separated from each other to a slight extent by intercellular edema. The peripheral cortical cells are normal in size and have small, round nuclei which stain less intensely than normally. The cells below this peripheral zone are normal in size and staining intensity.

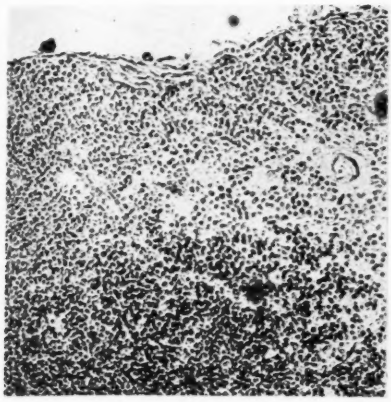
FIG. 7. Thymus (rabbit) exposed to phosphate-water for 1 hour at 38°C. × 350. The tissue has a granular and fibrillar precipitate which is basophilic in the stained section. The persisting cortical cells have slightly enlarged, deeply staining nuclei.

FIG. 8. Thymus (rabbit) exposed for 30 minutes at 38°C. to a mixture of 9 parts of phosphate-water and 1 part of phosphate-Ringer's solution. × 700. The figure shows a longitudinal section of a large blood vessel. The endothelial cells are enlarged with large, spindle-shaped nuclei. Beneath the endothelium is a layer of enlarged cells with clear, unstained cytoplasm. The round nuclei of the cells stain slightly. These cells presumably are smooth muscle cells of the muscularis. The outer layer of the blood vessel is composed of collagenous fibers.

FIG. 9. Thymus (rabbit) exposed to phosphate-Ringer's solution for 4 hours at 45°C. × 350. The cortical cells are sparse in number. The persisting cells are normal in size and have round, dark nuclei. The blood vessel in the upper part of the section has many fairly well preserved red blood cells and a few leukocytes.

FIG. 10. Spleen (rat) exposed to phosphate-water for 6 hours at 2°C. × 350. The tissue has a thick, fibrillar precipitate which is eosinophilic in the stained section. In the interstices of the fibrils are a few enlarged cells with clear cytoplasm and pale nuclei.

FIG. 11. A human, cervical lymph node treated with phosphate-water for 30 minutes at 38°C. × 350. The lymphocytes are markedly enlarged with clear, unstained cytoplasm. The cell walls are prominent. Some nuclei are normal in size and dark in color and others are enlarged and stain lightly.



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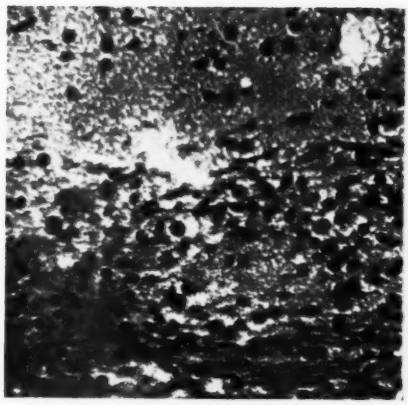

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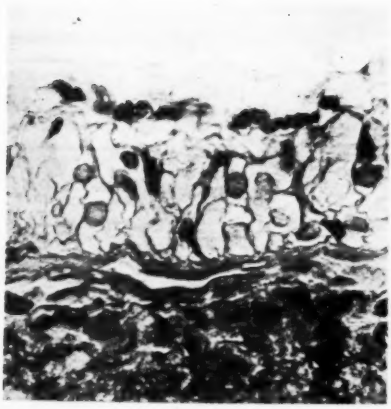

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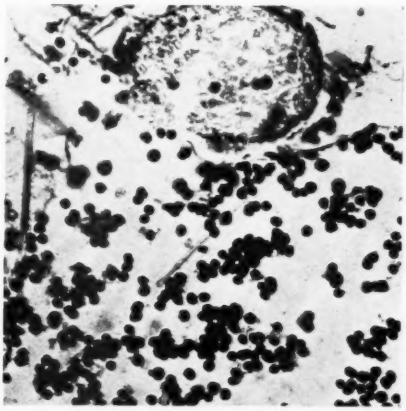

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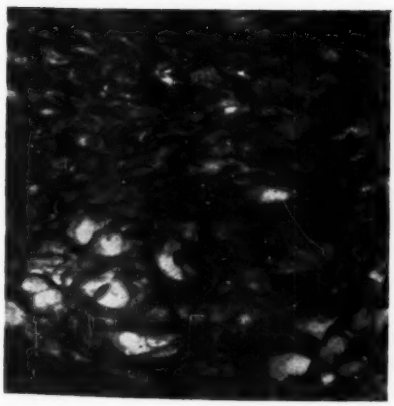

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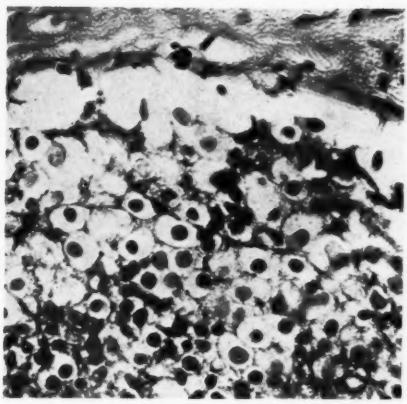

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PLATE 195

FIG. 12. A cervical lymph node of a rabbit. The node was exposed to distilled water for 5 hours at 2°C. × 350. In one area the cells are replaced by a fibrillar and granular precipitate. The persisting lymphocytes are enlarged. The cytoplasm of the cells is increased in volume and is unstained. The nuclei are normal in size and stain deeply.

FIG. 13. A para-aortic lymph node of a rabbit. The tissue was subjected to distilled water for 30 minutes at 38°C. × 350. The lymph follicle is composed of lymphocytes which are normal in size and in staining intensity. The medulla has few cells, both in this section and in control sections of untreated tissue.

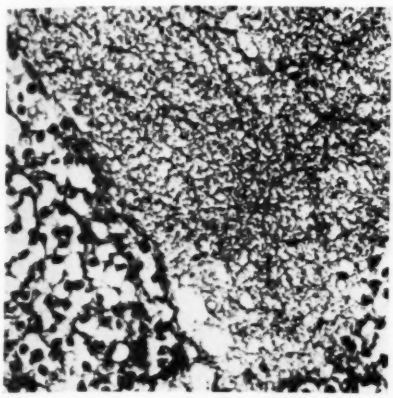
FIG. 14. Testis (rabbit) incubated for 30 minutes at 38°C. with a mixture of 9 parts of phosphate-water and 1 part of phosphate-Ringer's solution. × 350. The spermatogenic cells are enlarged, filling the tubule. The cytoplasm is unstained. The nuclei are very large and stain poorly. No mitotic figures are present.

FIG. 15. Testis (rabbit) incubated with phosphate-water at 38°C. for 30 minutes. × 350. The tubule is filled with amorphous eosinophilic débris. No spermatogenic cells can be seen. Many small, spindle-shaped, deeply stained nuclei of spermatocytes are still present in the tubule.

FIGS. 16 and 17. Testis (rat) treated with phosphate-water for 6 hours at 4°C.

FIG. 16. The fibrous framework of the tubules is well preserved. A few blood vessels are present in the fibrous tissue. The spermatogenic cells and the spermatozoa have disappeared, leaving empty spaces in the tubules. A small amount of amorphous detritus persists in a few tubules. × 120.

FIG. 17. The endothelial cells of the small arteriole are cuboidal and markedly enlarged. The cell walls are prominent and the cytoplasm is unstained. The nuclei are large, round, and lightly stained. The smooth muscle cells in the muscularis are not enlarged and have deeply staining cytoplasm and nuclei. There is some enlargement of the nuclei of these cells. × 700.



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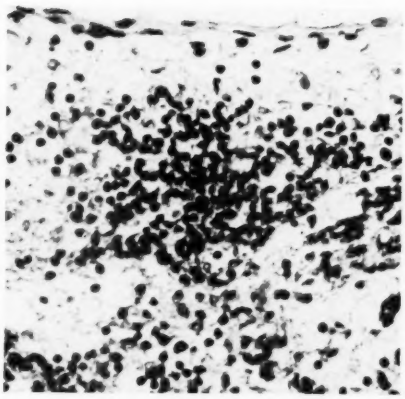

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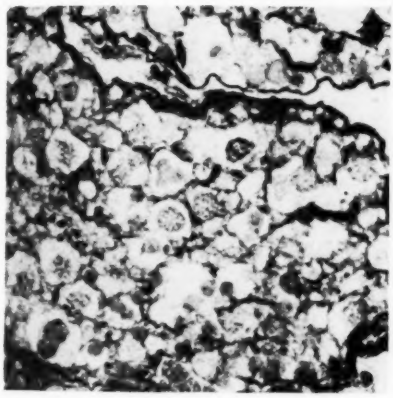

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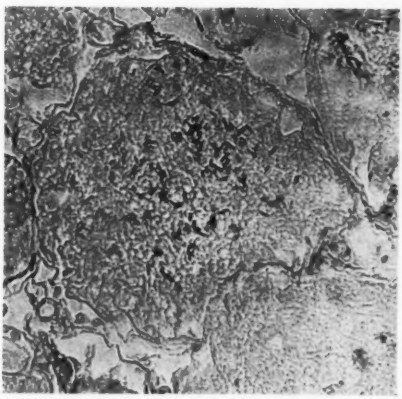

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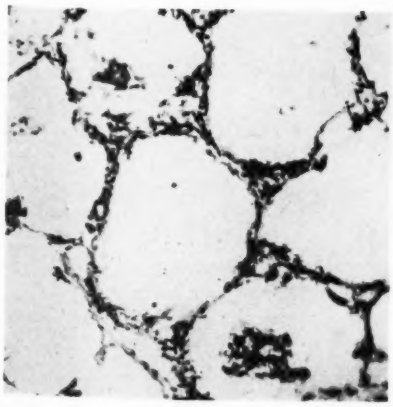

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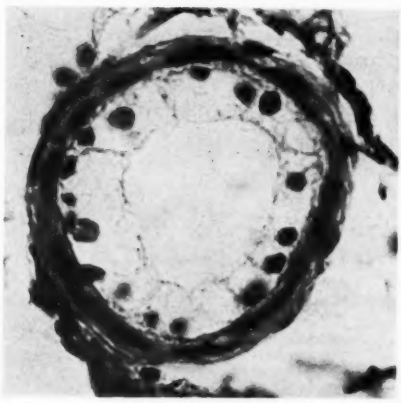

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Reactions to Hypotonic Solutions

PLATE 196

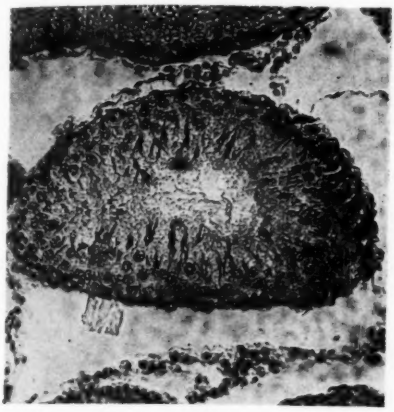
FIG. 18. Testis (rabbit) incubated with phosphate-Ringer's solution for 1 hour at 38°C. × 170. The cells in the tubules have separated from the basement membrane, probably due to intertubular edema. The cellular architecture of the tubules is well preserved. The cells are apparently normal in size, and the cytoplasm and nuclei stain deeply. Many cells are in mitotic division.

FIG. 19. Testis (rabbit) exposed to phosphate-sucrose solution (5 per cent phosphate buffer, pH 7.6, in a 0.33 molar solution of sucrose) for 3 hours at 2°C. × 250. The tubule has numerous, large, multinucleated cells with deeply staining cytoplasm. The cells have 2 to 10 fairly large nuclei which are vesicular in some cells and which stain deeply and uniformly in other cells.

FIG. 20. A normal salivary gland of a rabbit. × 350. The tissue was fixed immediately after excision. The blood vessel beneath the duct is filled with red blood cells.

FIG. 21. Salivary gland of a rabbit. The tissue was incubated with phosphate-water for 1 hour at 38°C. × 350. The cells in the tubule are not enlarged. The cytoplasm stains intensely. The nuclei are normal in size and stain deeply and uniformly. A small arteriole below the duct has enlarged endothelial cells and does not contain any blood cells. The periductal fibrous tissue is edematous. The acinar cells are normal in size. The cytoplasm is granular as in the control section. The nuclei are somewhat smaller than normal, slightly irregular, and stain deeply.

FIG. 22. Transplantable leukemic myeloblastoma grown as a subcutaneous nodule in a mouse. The tumor was incubated with phosphate-water for 30 minutes at 38°C. × 350. The section has three distinct zones. The peripheral zone at the right is composed of amorphous, eosinophilic débris with an occasional, persisting nucleus. These features result from the experimental procedure. A second layer is composed of enlarged cells with clear cytoplasm and enlarged, lightly staining nuclei. The third layer is composed of leukemic cells which are normal in appearance.



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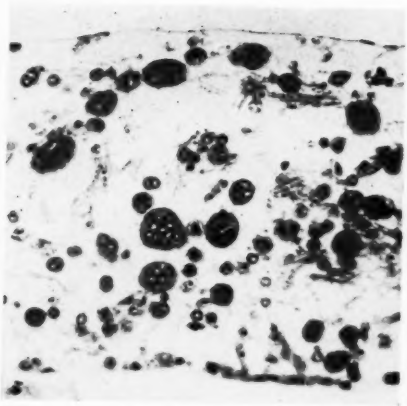

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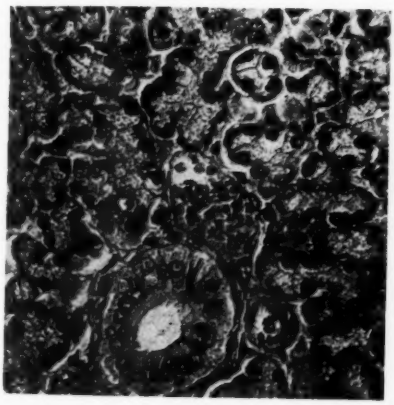

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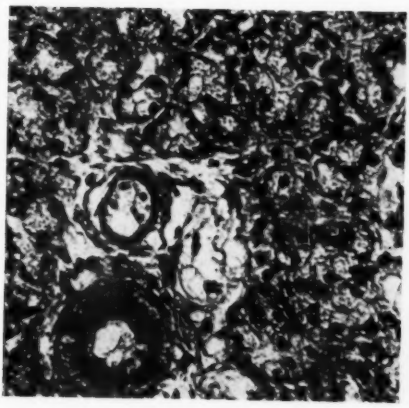

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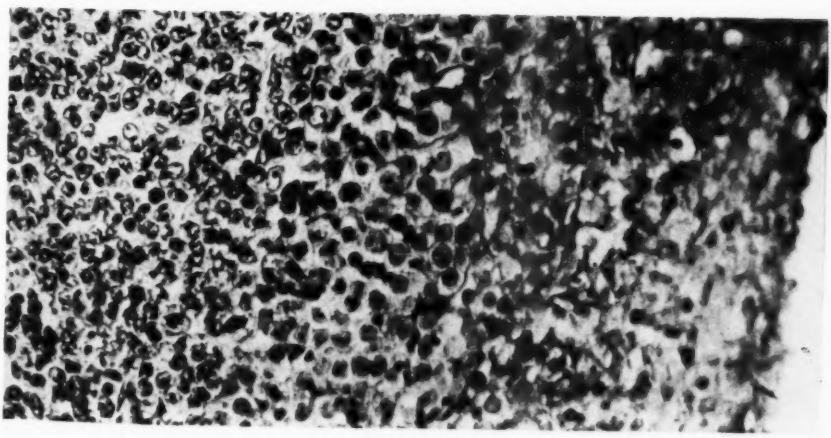

22

A PATHOLOGICAL STUDY OF MICE INFECTED WITH THE VIRUS OF SWINE INFLUENZA *

I. N. DUBIN, M.D.†

(From the Department of Pathology, Duke University School of Medicine, Durham, N. C.)

Since the demonstration by Andrewes, Laidlaw, and Smith[1] of the susceptibility of mice to the viruses of human and swine influenza, white mice have been used extensively in the study of these diseases. Several detailed reports [2-7] of the lesions produced in mice by infection with the virus of human influenza have been presented. On the other hand I have been able to find only one paper dealing with the detailed histopathology of the infection in mice by the virus of swine influenza. In that paper Straub [5] described the lesions produced in the respiratory tract by the viruses of both human and swine influenza. The changes were apparently identical and were described together. Both viruses affected specifically the epithelium of the respiratory tract from the bifurcation of the trachea to the bronchioles, producing a necrosis followed by proliferation and regeneration of the epithelium. Catarrhal bronchitis was followed by collapse of the lung tissue. Straub stated that the pulmonary changes were secondary to the bronchitis and that there was no real pneumonia. He described only the bronchi and lungs and made no mention of lesions in other organs.

Because only the bronchi and lungs have heretofore been described, the present experiment was done in order to make complete anatomical studies of mice receiving intranasal inoculation of the virus of swine influenza.

MATERIALS AND METHODS

White mice, 4 to 5 weeks old, were used. The virus of swine influenza, obtained from Dr. J. W. Beard, had been passed several times in chicken embryos and was received in the form of a bacteria-free suspension in chorioallantoic fluid.

After the mice were anesthetized with ether, 0.05 cc. of the virus suspension was introduced into the nose with a tuberculin syringe. There were two test groups. One group received the undiluted suspension of virus in chorioallantoic fluid. Of this group, 74 were examined microscopically after a period of infection ranging from 6 hours to 35 days. The potency of this suspension was such that the 50 per cent end point [8] for gross pulmonary lesions was a dilution of $10^{-7.7}$. In the other group the inoculum consisted of a 10 per cent suspension

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† Now at the Institute of Pathology, University of Tennessee, Memphis 3, Tennessee.

in broth of lungs from mice previously inoculated with the virus. Cultures of this suspension showed a light growth of *Bacillus coli* and nonhemolytic streptococci. As will be seen later, these bacteria were of no significance in the development of the lesions. Of this second group, 73 mice were examined microscopically at intervals ranging from 6 hours to 28 days. The 50 per cent end point for gross pulmonary lesions of this inoculum was a dilution of $10^{-5.4}$.

Controls consisted of 23 untreated mice, 19 mice subjected only to ether anesthesia, 26 mice receiving intranasal inoculation of normal mouse lung, and 20 mice receiving intranasal inoculation of normal chorioallantoic fluid. These animals were killed at intervals ranging from a few hours to 28 days.

Because small numbers of bacteria were present in the virus-lung inoculum and because similar bacteria were later cultured from the lungs of some of the test animals, a further control experiment was done in order to determine whether intranasal inoculation of the bacteria alone could produce pneumonia in mice. A Salmonella and a strain of *B. coli* were isolated from the lungs of mice which had been inoculated with the virus of swine influenza. Forty-eight hour cultures of the bacteria grown on Douglas agar slants were suspended in physiological saline solution so that 1 cc. of the suspension contained approximately one-half billion organisms as determined by turbidity standards. In addition, a 1:200 dilution of each of the two suspensions (Salmonella and *B. coli*) was made. Of these four bacterial suspensions, 0.05 cc. was inoculated intranasally after ether anesthesia, using four groups of mice. One group of 8 mice received approximately 25 million Salmonella organisms. Another group of 7 mice received about 125,000 Salmonella organisms. Two other groups of 7 mice each received like numbers of *B. coli* organisms respectively. A fifth group of 5 mice was given sterile saline washings of Douglas agar slants. All mice in these five groups survived the inoculation and were killed on the fourth day. The lungs from about one-half of them were cultured.

As an additional check on the identity of the infecting agent in the test animals, protection tests were made with serum from swine vaccinated with formalin-inactivated virus of swine influenza. A mixture of immune swine serum and virus produced no lesions in mice, while a mixture of normal swine serum and virus produced gross pulmonary lesions.

Complete autopsies were performed shortly after death on animals that died. Other animals were killed with ether and examined immediately. In some cases the lungs were removed under sterile conditions and cultures made. The tissues were fixed in either Zenker-

acetic or Zenker-formalin solutions. After the skull was decalcified, sections were made of the nose and nasopharynx. Sections for microscopical study were made from paraffin blocks and were stained with hematoxylin and eosin and with MacCallum's bacterial stain.[9]

Results

Clinical. Symptoms of infection appeared 1 to 3 days after inoculation of the virus. The mice became comparatively inactive and ate and drank less. The fur became ruffled. Respiratory movements were exaggerated and breathing was labored. Most deaths occurred between the 2nd and 5th days, the maximum number occurring on the 4th day. Animals died as early as 18 hours and as late as 9 days after inoculation of the virus. All mice that died had gross pulmonary lesions.

Bacteriological. In the group of mice which received the virus-egg suspension, cultures of the lung showed a very light growth of *B. coli* on the 1st day and were negative thereafter. No bacteria were seen in sections. The presence of both gram-positive and gram-negative bacilli in the intestine on the same slides as the lungs served as a control for the stain. In the group of mice which had received the virus-lung suspension, cultures of the lungs showed a very light growth of *B. coli* and α-streptococci in the first 48 hours and were negative thereafter. No bacteria, however, were seen in sections.

Cultures of the lungs from the mice which received the bacterial suspensions were negative in about half the cases and in the other half yielded a light growth of the inoculated organism. No growth was obtained from cultures made of the lungs of mice inoculated with the washings of agar slants. No bacteria were seen in the specially stained sections of lungs of any of these groups. Apparently too few of these gram-negative organisms remained in the lung on the 4th day to be discoverable in the sections.

Cultures of lungs from the remaining control groups were all negative for bacteria; no bacteria were seen in sections.

Pathological Anatomy of the Two Groups Receiving the Virus

Gross Lesions

Except for rare and minimal changes in other organs, all important lesions were confined to the lower part of the trachea, the bronchi, and the lungs. The anatomical changes were, with the exception of difference in severity of alveolar necrosis, identical in both groups of mice, and will be described together.

All mice that died had gross pulmonary lesions. The mouse that died in 18 hours showed consolidation of approximately 10 per cent of

the pulmonary tissue. All mice that died subsequently had gross involvement of at least 70 per cent of the pulmonary tissue. The solidified areas were of a bluish purple, homogeneous appearance. The lungs were relatively airless and sank in the fixing fluid. When smaller areas of the lungs were involved, these tended to be localized to the hilus and posteriorly. In the first few days the affected regions were quite wet but later became dry.

Microscopical Lesions

At 6 Hours. Lesions were found in 3 of 7 animals. The lower portion of the trachea contained a few polymorphonuclear leukocytes; the tracheal epithelium did not appear abnormal. The larger bronchi were filled with pus, and many polymorphonuclear leukocytes were present in all coats. The bronchial epithelium showed no evidence of necrosis at this time. The pulmonary lesions were very slight and restricted to small areas around the bronchi. These areas showed interstitial infiltration by many polymorphonuclear leukocytes and a small number of mononuclear cells. The alveoli were empty. One outstanding feature was a marked edema of the lymphatics around the larger bronchi and vessels.

At 18 to 24 Hours. Lesions were present in 6 of 7 animals. One died at 18 hours. The tracheal epithelium showed slight focal necrosis and was covered by a coat of polymorphonuclear leukocytes. The submucosa also contained leukocytes. Pus was present in the bronchi, bronchioles, and regional alveolar ducts. There was increased secretion of mucus by the bronchial epithelium. There was now definite evidence of necrosis and desquamation of the bronchial and bronchiolar epithelium (Fig. 2). Necrosis of the cells lining the alveoli was present in a few foci. The walls of the bronchi and bronchioles contained many polymorphonuclear leukocytes. Occasionally a bronchus was so plugged with pus that collapse of the corresponding portion of the lung resulted; this, however, was not a prominent feature at this stage. The lesion was still mainly bronchial. The alveoli for the most part were empty and the interstitial reaction was slight; polymorphonuclear leukocytes predominated although mononuclear cells also were present. The dilatation of the peribronchial and perivascular spaces was quite marked. An occasional arteriole and vein showed penetration of all coats by small numbers of polymorphonuclear leukocytes.

At 2 Days. Changes in the trachea reached a maximum 2 days after infection, but were only slight compared to those of the bronchi and alveoli. In a few areas the tracheal epithelium consisted of a single

layer of flat cells. Elsewhere, hyperplasia of the tracheal epithelium was in progress (Fig. 9).

Necrosis of the bronchial and bronchiolar epithelium was considerable, and that of the lining cells of the alveoli moderate. In some areas the epithelium of the bronchi and bronchioles was completely denuded; in others there was a single layer of flat epithelium. The necrotic lining often appeared as a pink hyaline membrane. Many small bronchi were now stuffed with pus and atelectasis was prominent. The interstitial exudate was now more diffuse and contained more cells, with polymorphonuclear cells predominating. The polymorphonuclear leukocytes were at their maximum number on this day. Mononuclear cells began to appear in moderate numbers in the bronchial wall, the lymphatics, and the interstitial exudate. The alveoli contained fluid, mainly, and small numbers of polymorphonuclear leukocytes, mononuclear cells, desquamated alveolar lining cells, and débris; there was no fibrin. In the mice that died there was marked congestion and some hemorrhage as well as a considerable degree of edema. The lymphatics were still prominent and contained more cells (Fig. 1).

At 3 Days. Three days after infection the necrotic process in the tracheal epithelium had just about disappeared, while a slight degree of hyperplasia persisted. Necrosis of the lining of the bronchi and alveoli had almost reached its peak. Numerous examples of a hyaline membrane were seen in bronchioles, alveolar ducts, and alveoli proper. Many bronchi and bronchioles were stripped clean of epithelium, while others showed a single layer of flat cells (Fig. 8). The interstitial cells were more prominent now, with polymorphonuclear and mononuclear cells about equal in number. There was more congestion, focal hemorrhage, and edema than on the 2nd day. In the mice that died spontaneously the interstitial cellular reaction was less, while congestion, edema, and hyaline necrosis of the epithelium were all more prominent than in the mice that were killed. The bronchial epithelium was beginning to show signs of regeneration in the form of a single layer of large polymorphic cells.

At 4 Days. The necrotic process of the bronchial epithelium was at its worst 4 days after infection and that of the alveolar cells followed closely. The necrotic lining was often seen as a cast which had fallen away from the wall. Proliferation of the epithelium of the larger bronchi was present to a moderate degree, producing two or three layers of cells (Fig. 10). These cells had large, dark nuclei with prominent nucleoli; some mitotic figures were seen. The arrangement of the nuclear material produced no uniform pattern. No inclusion

bodies were identified. A lesser degree of proliferation was seen in the smaller bronchi and bronchioles. In addition there was a slight proliferation of the cells lining the alveoli resulting in small clumps of three or four cells; this was present as much at the periphery of the lung as at the hilus and one received the impression that it was the result of multiplication of alveolar lining cells themselves rather than of downgrowth of cells from the bronchioles. The latter process was to become prominent later in the disease. The number of cells in the interstitial tissues had increased, with mononuclear cells about twice as numerous as polymorphonuclear cells. The animals that died showed more necrosis and less proliferation of the lining cells of the bronchi and alveoli than those that were sacrificed.

At 5 Days and Beyond. The proliferation of the tracheal epithelium, never more than slight, was absent by the 7th day.

The necrotic process in the bronchial epithelium was still marked on the 5th day but rapidly decreased thereafter, being absent entirely by the 7th day (Figs. 4 to 7). In the animals which received the virus-lung suspension, alveolar necrosis reached its maximum intensity on the 4th and 5th days and was gone by the 8th day. In the other group (virus-egg suspension), alveolar necrosis was less severe and proceeded more slowly, reaching a peak between the 6th and 8th days and disappearing entirely by the 10th day.

The proliferative process proceeded at different rates in the large bronchi, small bronchi, and alveoli. In the large bronchi hyperplasia was well marked on the 5th day, reached its maximum on the 6th and 7th days, and disappeared between the 11th and 17th days. In the smaller bronchi and bronchioles hyperplasia reached its maximum intensity between the 8th and 10th days and disappeared between the 14th and 20th days (Figs. 11 and 12). Many of the superficial cells of the proliferating epithelium underwent desquamation. When the proliferative process in the bronchial epithelium had disappeared, the cells appeared more or less normal, showing restoration of cilia and return of the nucleus to the base of the cell.

The proliferation in the bronchioles resulted in the formation of solid clumps of epithelial cells which at first remained confined to the bronchiolar lumen but soon extended into the alveoli (Figs. 13 and 14). The latter process was present to a moderate degree on the 7th day and reached its maximum between the 10th and 12th days. The peripheral portions of the lungs which had not as yet been invaded by this process still showed proliferation of the lining cells of the alveoli. Soon these two hyperplastic processes fused. The solid plugs of cells often underwent squamous metaplasia, and intercellular bridges

could be identified. It seemed that many of these occluded alveoli opened up again to some extent as a result of degeneration of the epithelial plugs. About the 14th day the nuclei of the cells forming the plugs became swollen and pale and the prominent dark clumps of chromatin were replaced by fine, pale granules (Fig. 15). In other plugs, the center contained hyaline masses of necrotic cells. Eventually the alveoli were lined by a single layer of cuboidal epithelium. The central hyaline masses were surrounded by polymorphonuclear leukocytes (Figs. 16 and 17). These leukocytes, which had almost disappeared by the 5th day, returned in small numbers but were restricted to the lumina of those alveoli which contained this hyaline débris. The process of degeneration of the solid plugs and partial reopening of the alveoli was well established by the end of the 3rd week. At this time the lung looked like a gland, the alveoli being lined by tall epithelial cells. This appearance was still present at 5 weeks, the end point of this experiment. Even though some alveoli were thus reopened, the affected portions of the lung were probably of little use in aeration of the blood since the alveolar lumina were small and the interstitial tissues considerably thickened as a result of infiltration by mononuclear cells. Moreover, many parts of the lungs were completely collapsed and solid.

In the meantime, the edema of both the alveolar lumina and the lymphatics was decreasing and was much less by the 10th day. Interstitial mononuclear infiltration was prominent on the 5th day and was at its height between the 6th and 8th days. Thereafter it decreased slowly and was still present in a slight to moderate degree at the end of the 5th week.

Differences Between Group Receiving Virus-Egg Suspension and Group Receiving Virus-Lung Suspension. Two differences were seen between the group of mice receiving the virus-egg suspension and the group receiving the virus-lung suspension. In the latter the necrosis of the alveolar lining cells was more extensive and occurred earlier in the disease. In addition, although the exact mortality rates were not established since some animals were killed that were obviously moribund, the mortality rate in the virus-lung group was about twice that in the other.

Changes in Other Organs. Changes other than in the lungs were rare. In the first few days after infection the mediastinal lymph nodes showed distention of the sinusoids with fluid as well as with many macrophages and a few polymorphonuclear leukocytes. In one mouse killed at 5 days there were several small perivascular collections of mononuclear cells in the myocardium. In one mouse which died at 5

days an artery near the epididymis showed infiltration by polymorphonuclear leukocytes. Slight leukocytic infiltration of one adrenal was present in one mouse killed on the 7th day. Two other findings of common occurrence were present in the test and control groups with equal frequency: Mucus and pus in the nose, and focal collections of mononuclear cells and leukocytes in the liver.

PATHOLOGICAL ANATOMY OF CONTROL GROUPS

Control Groups Receiving Bacterial Suspensions. Of 8 mice which received the undiluted suspension of Salmonella, 4 were normal and 4 showed a slight degree of interstitial mononuclear pneumonia. In the latter the bronchial epithelium was normal except for an increased secretion of mucus. The alveolar lumina were empty. Small numbers of mononuclear cells and fewer polymorphonuclear leukocytes were present in the interstitial tissues of the lung. No other abnormalities were seen. There was nothing comparable to the severe bronchitis and pneumonia produced by the virus. Of 7 mice which received the diluted suspension of Salmonella, only 2 showed a slight degree of interstitial reaction; the other 5 were normal. In the group of 7 mice which received the undiluted suspension of *B. coli,* 3 were normal and 4 showed an interstitial mononuclear response similar to that elicited by the Salmonella. Of 7 mice which received the diluted suspension of *B. coli,* only 1 showed any degree of interstitial reaction. Of the 5 mice which were inoculated with the agar washings, 3 were normal and 2 showed a minimal cellular infiltration of the peribronchial interstitial tissues.

Remaining Control Groups. The mice in the remaining control groups showed no lesions except for slight leukocytic infiltration in the interstitial tissues of the lung. In a few animals small collections of polymorphonuclear leukocytes were seen in the lumina of the bronchi in the first few days.

DISCUSSION

The lesion produced in mice by infection with the virus of swine influenza consisted essentially of necrosis of the epithelium of the bronchi and bronchioles, commonly producing hyaline membranes, together with a marked reparative proliferation of this epithelium resulting finally in restoration of the normal lining epithelium. In the bronchioles the proliferative process was so active that the hyperplastic epithelium grew into and filled the regional alveoli. The hyperplasia began shortly after the onset of necrosis and the two processes,

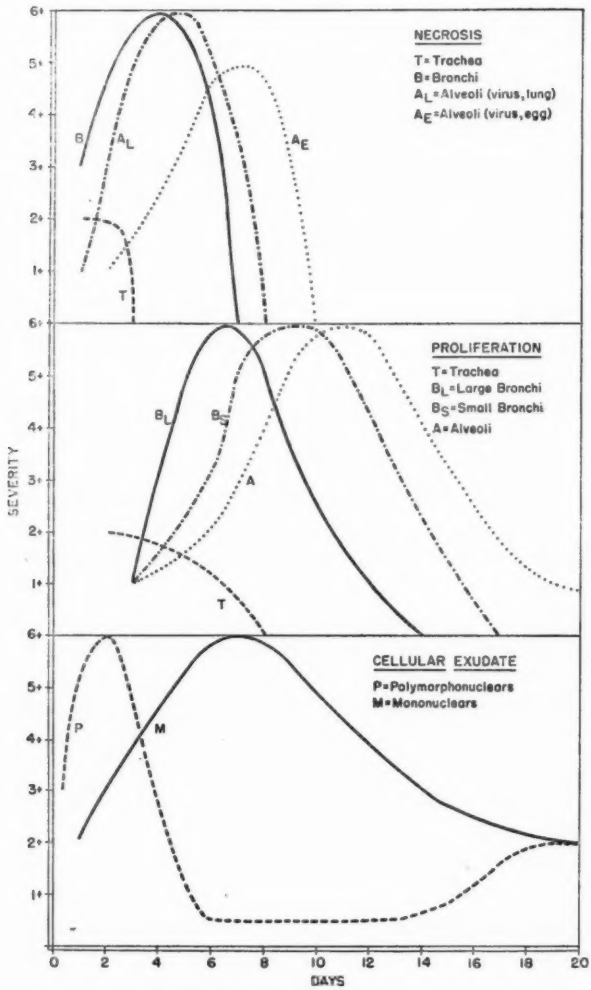
necrosis and proliferation, went on side by side. A similar lesion, although of much less intensity, occurred in the lower portion of the trachea. In addition to bronchitis there was also a pneumonia characterized by a hyaline necrosis of the alveolar lining and an interstitial exudate which was predominantly polymorphonuclear in the first 2 days and mononuclear thereafter. The pneumonia was further complicated by large areas of atelectasis which appeared as early as the 2nd day and resulted from obstruction of the bronchi by exudate, mucus, and desquamating epithelium. Deaths occurred mainly on the 4th day and were apparently caused by two main factors, asphyxia and viral intoxication. There were three factors involved in the production of asphyxia: (1) atelectasis; (2) destruction of the alveolar lining cells with formation of a hyaline membrane; (3) extensive capillary damage, resulting in severe congestion, focal hemorrhage, and edema of such a degree that some animals literally drowned in their own fluid. The factor of viral intoxication was difficult to judge; one animal died in 18 hours with slight involvement of the lungs, but all other animals that died of the disease had extensive pulmonary lesions.

The experiment is summarized in Text-Figure 1 consisting of three graphs in which the processes of necrosis, proliferation, and cellular exudate are plotted against time. The abscissas show the number of days after inoculation of the virus, while the ordinates indicate the severity of the process, ranging from 1 plus to 6 plus. The severity was measured subjectively. Therefore the curves are to be considered as diagrammatic and as representing trends rather than exact mathematical replicas of the processes.

The formation of the pink-staining hyaline membranes in the bronchioles and alveoli was clearly on the basis of necrosis of the lining cells. The membranes were formed by a fusion of these necrotic cells and one could often see nuclear remnants within them (Figs. 4 to 7). They could hardly have been produced by the packing of fibrin into the alveoli since there was no fibrin in the alveolar exudate.

As to the etiology of the pneumonia, it seems quite clear that the only significant etiological agent was the virus itself. The few bacteria introduced into the lungs, either by their presence in the inoculum in one case (virus-lung suspension) or by their being washed down from the nasopharynx in the other (virus-egg suspension), played no part either in the pathogenesis of the pneumonia at the onset or in the production of a secondary bacterial pneumonia. In the former group, bacteria were cultured from the lungs in the first 48 hours only, and, in the

latter, in the first 24 hours only. Thereafter, the cultures of the lungs were negative and the pneumonia progressed to its peak on the 4th day in the absence of bacteria. No bacteria were seen in the specially stained sections of lung in either group. It was seen that the intranasal inoculation of about 25 million Salmonella or *B. coli* organisms produced only



Text-Fig. 1. The three main processes of epithelial necrosis, epithelial proliferation, and cellular exudation are represented in these graphs. The abscissas show the number of days after inoculation of the virus and the ordinates the estimated intensity of the processes. These curves are purely diagrammatic.

a slight degree of interstitial mononuclear reaction in the lungs; it is quite improbable that such a large number of bacteria were washed down with the virus suspension. Although small numbers of nonhemolytic streptococci were present in the virus-lung inoculum, it seems reasonably certain that they were of no importance in the pathogenesis of the pneumonia, since no cocci were seen in the sections of lung stained for bacteria.

The lesions described in this experiment are essentially similar to those produced in mice by Straub [4, 5] with the viruses of swine and human influenza, and by Nelson and Oliphant [6] with virus A of human influenza and Oliphant and Perrin [7] with virus B of human influenza. Straub described the bronchi and lungs only, while the others performed complete autopsies on the mice and found no important lesions outside of the trachea, bronchi, and lungs. In the present experiment, also, lesions were restricted to the trachea, bronchi, and lungs.

The nose of the mouse, unlike that of the ferret,[10] suffers no lesions from the introduction of the viruses of human and swine influenza.

No lesions were found in the brain in this experiment. Nelson and Oliphant [6] and Oliphant and Perrin [7] also found no lesions in the brains of mice given intranasal inoculations of the viruses of human influenza. Sheftel,[11] on the other hand, described changes in the brains of mice receiving intranasal inoculations of the Leningrad strain of the virus of human influenza.

The anatomical changes in the respiratory tract of mice, produced by the viruses of swine and human influenza, are quite similar to those of certain types of pandemic influenza-pneumonia in man described by Winternitz, Wason, and McNamara [12] and by Goodpasture,[13] and considered by Goodpasture probably to be of viral origin.

There has been considerable controversy as to whether the epithelial lining of the alveoli in chronic pneumonias is derived from the bronchioles or from the alveoli. In the present experiment I have gained the impression that the filling of the alveoli with hyperplastic epithelium was mainly the result of the downgrowth of such epithelium from the bronchioles. At the same time there was a slight degree of proliferation of the cells lining the alveoli. Whether or not these cells were epithelial in nature was not determined; at least they closely resembled the hyperplastic cells in the bronchi and they did not appear to be phagocytic.

Many of the solid plugs of epithelium in the alveoli underwent degeneration between the 2nd and 3rd weeks, resulting in partial reopening of some alveoli. On the other hand, Straub [5] has shown that some of these epithelial plugs persist for a year or even longer.

Summary

The intranasal inoculation of mice with the virus of swine influenza produced necrosis of the lining of the bronchi and alveoli and to a lesser extent of the lower portion of the trachea. The necrotic epithelium often appeared in the form of a hyaline membrane. Even before the necrotic process had ended, proliferation of the epithelium began and reached a remarkable degree in the bronchi and bronchioles. From the latter the proliferating epithelium invaded and filled the alveoli. Many of these intra-alveolar plugs of epithelium apparently began to degenerate about the 14th day and some alveoli were thus partially reopened. The epithelium of the bronchi and bronchioles was restored to normal sometime during the 3rd week.

In addition to bronchitis, there was a pneumonia characterized by hyaline necrosis of alveolar cells, congestion with focal hemorrhage, marked edema, and interstitial infiltration of inflammatory cells, chiefly mononuclear cells. Large areas of lung collapsed as early as the 2nd day, subsequent to obstruction of bronchi by pus, mucus, and desquamated cells. Most deaths occurred on the 4th day, mainly from asphyxia resulting from the pulmonary changes.

Lesions were restricted to the trachea, bronchi, and lungs. In particular, no changes were found in the nose or brain.

The lesions were essentially similar to those reported by others as occurring in mice following the intranasal inoculation of the virus of human influenza.

The photomicrographs were made by Mr. Carl M. Bishop.

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[*Illustrations follow*]

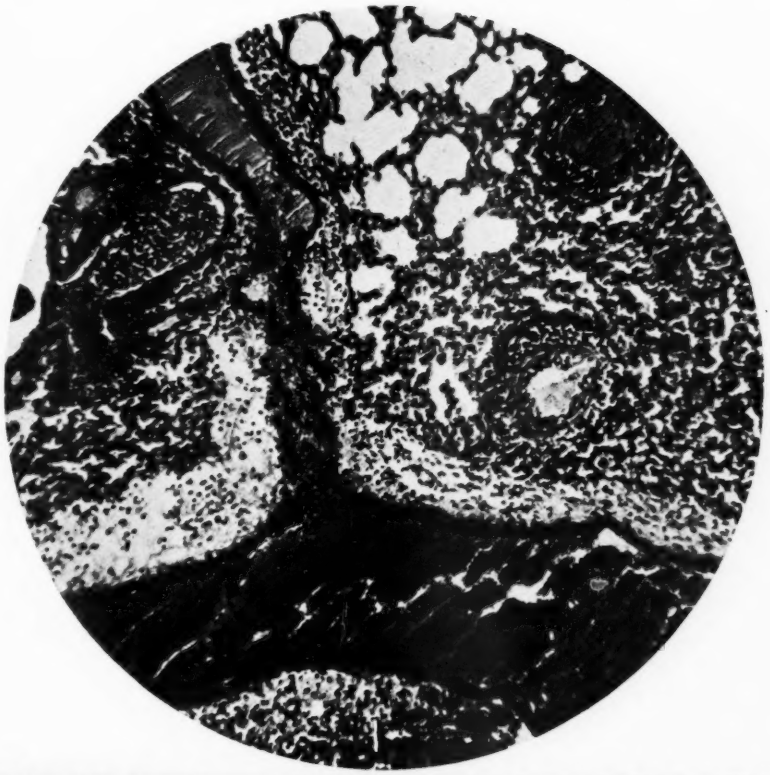
DESCRIPTION OF PLATES

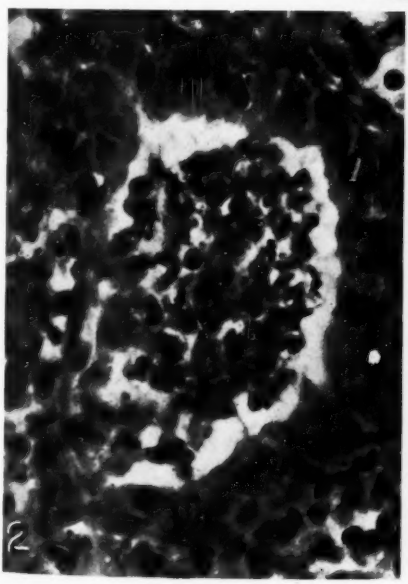
PLATE 197

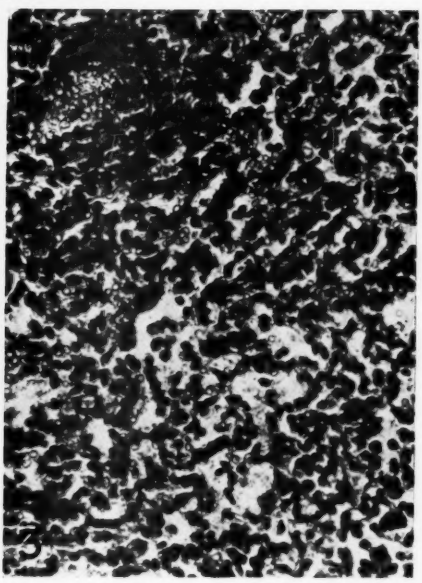
FIG. 1. Microscopical appearance of the lung of a mouse 2 days after intranasal inoculation of the virus of swine influenza. A bronchus in the upper left portion of the field is plugged with exudate; it is such occlusion of bronchi which leads to atelectasis, an area of which is seen at the extreme right. Also of note are the perivascular edema and cellular infiltration. Hematoxylin and eosin stain. × 122.

FIG. 2. Twenty-four hours after inoculation of the virus, the lumen of a small bronchus is filled with pus and desquamated necrotic epithelium. Hematoxylin and eosin stain. × 684.

FIG. 3. Diffuse interstitial mononuclear infiltration of atelectatic lung on the 5th day. Hematoxylin and eosin stain. × 265.







Dubin

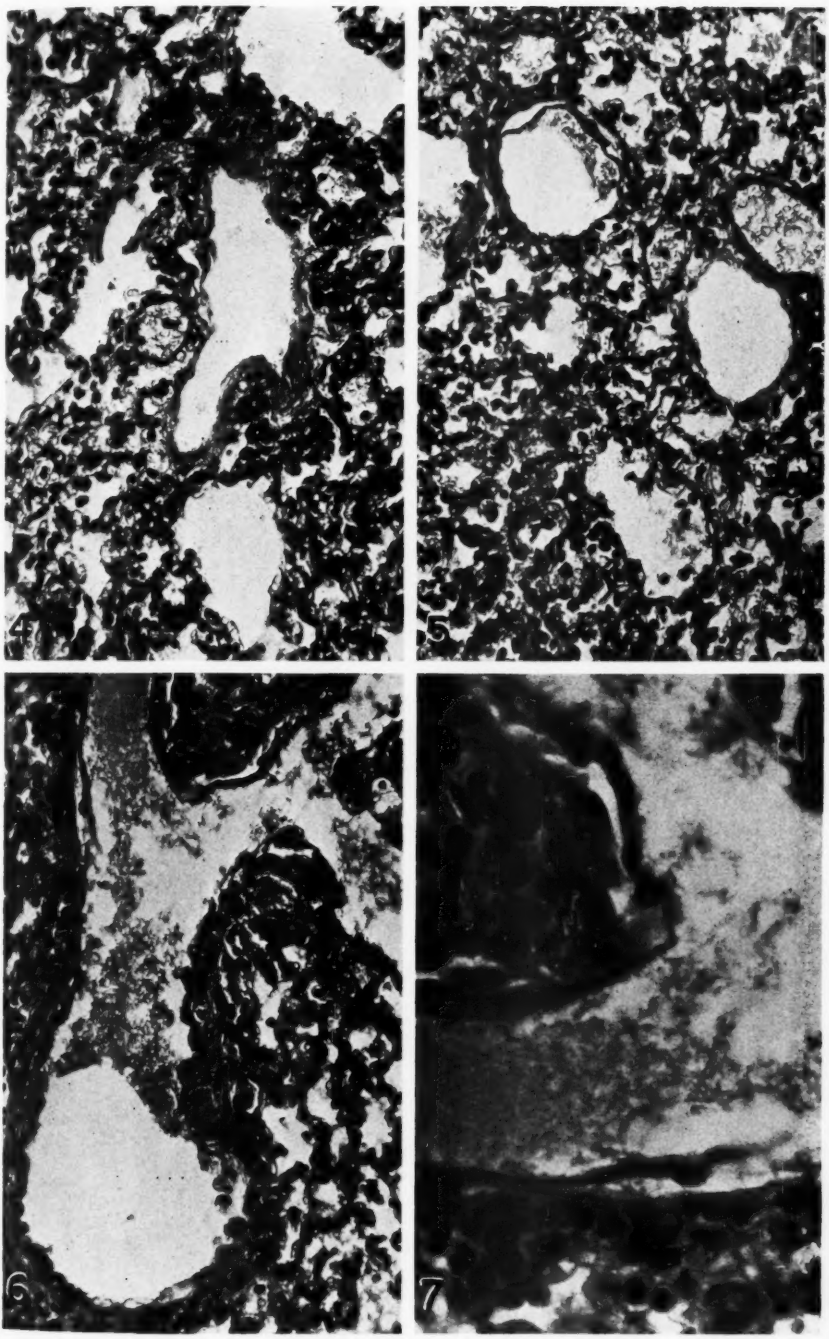
Mice Infected with Swine Influenza

PLATE 198

FIGS. 4 and 5. Hyaline membranes lining bronchioles, alveolar ducts, and alveoli; 6th day. Hematoxylin and eosin stain. × 275.

FIG. 6. Hyaline membranes in three adjacent alveolar ducts arising from one bronchiole. Damaged cells with pyknotic nuclei make up the membranes. The bronchiole is lined by a single layer of hyperplastic polymorphic cells; 5th day. Hematoxylin and eosin stain. × 265.

FIG. 7. High-power view of a portion of Figure 6 showing the formation of the membrane from necrotic epithelium. Hematoxylin and eosin stain. × 684.



Dubin

Mice Infected with Swine Influenza

PLATE 199

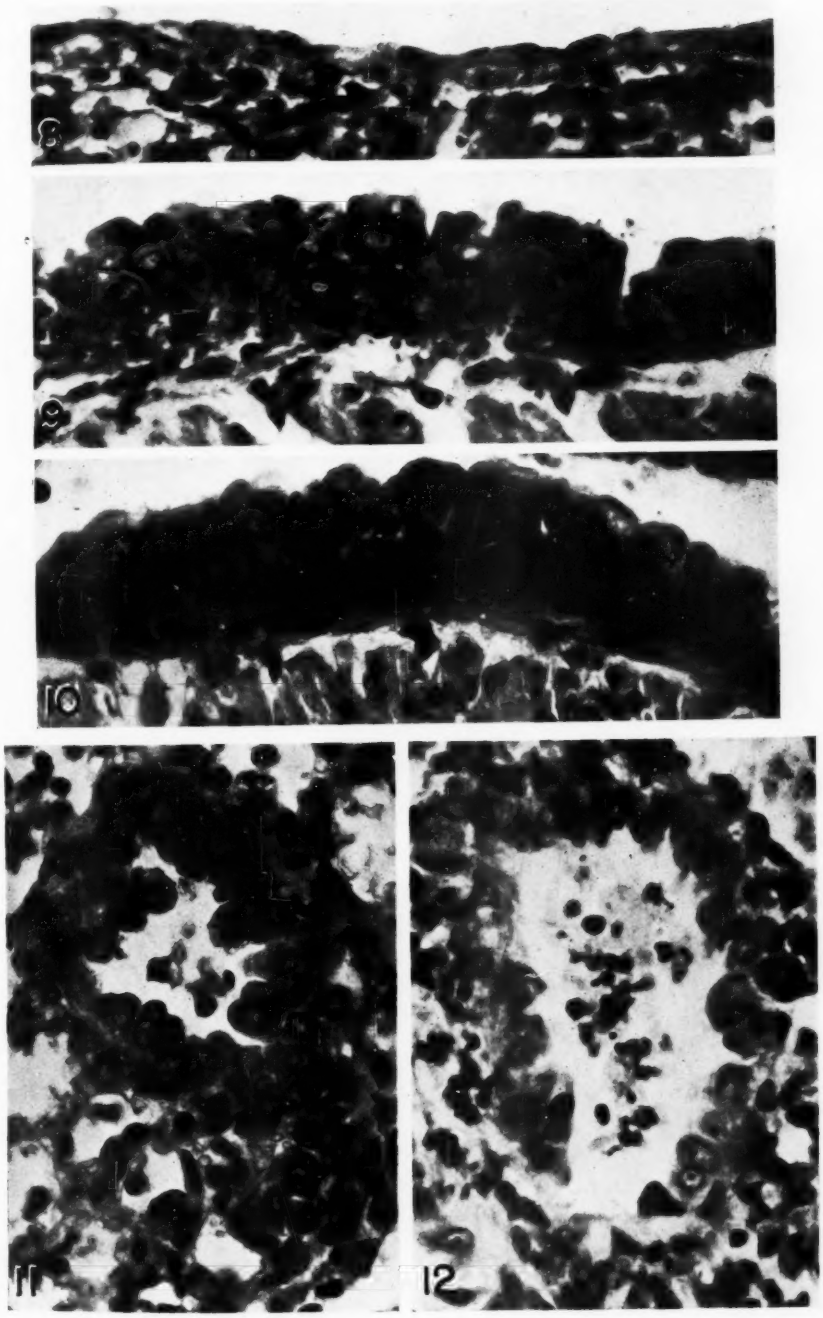
FIG. 8. Three days after inoculation. A large bronchus is now lined by a single layer of flat cells. Hematoxylin and eosin stain. × 684.

FIG. 9. Two days after inoculation. Proliferation of the lining epithelium of the trachea. In most instances the trachea did not show as much proliferation as this. Hematoxylin and eosin stain. × 684.

FIG. 10. Four days after inoculation. Proliferation of the lining epithelium of a large bronchus. Hematoxylin and eosin stain. × 684.

FIG. 11. Five days after inoculation. Proliferation of the epithelium of a bronchiole. Hematoxylin and eosin stain. × 684.

FIG. 12. Seven days after inoculation. A small bronchus is lined by hyperplastic epithelium and its lumen still contains débris. Hematoxylin and eosin stain. × 684.



Dubin Mice Infected with Swine Influenza

PLATE 200

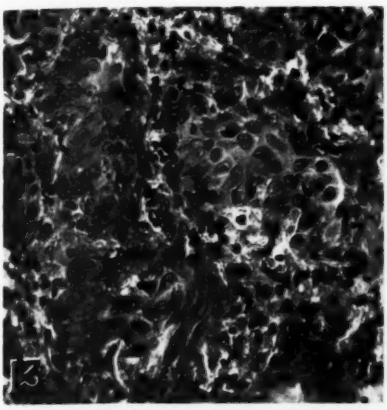
FIG. 13. Twelve days after inoculation. The alveoli are filled with epithelial plugs, some of which resemble squamous epithelium. Hematoxylin and eosin stain. × 265.

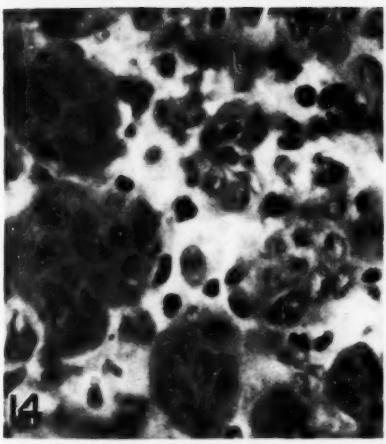
FIG. 14. High-power view of a field adjacent to that seen in Figure 13. Hematoxylin and eosin stain. × 684.

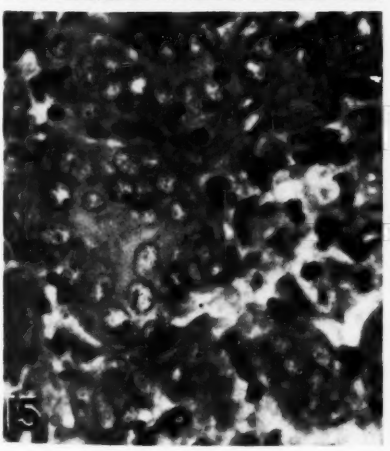
FIG. 15. Fourteen days after inoculation. The nuclei of the cells forming the epithelial plugs are swollen. This will be followed by necrosis and desquamation of these cells and by partial reopening of some alveoli. The appearance of these cells may be compared with those in Figure 14 photographed at the same magnification. Hematoxylin and eosin stain. × 684.

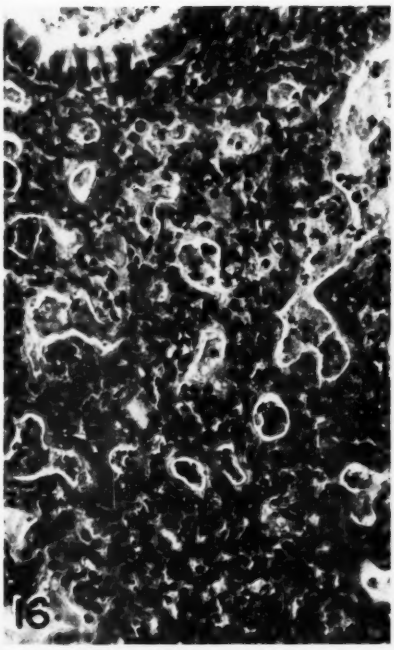
FIG. 16. Nineteen days after inoculation. The alveolar ducts and alveoli are filled with hyaline eosinophilic masses consisting of degenerated epithelial cells. The interstitial tissue contains mononuclear cells. The bronchus in the upper left portion of the field still contains fluid and débris; the epithelium is relatively normal. Hematoxylin and eosin stain. × 265.

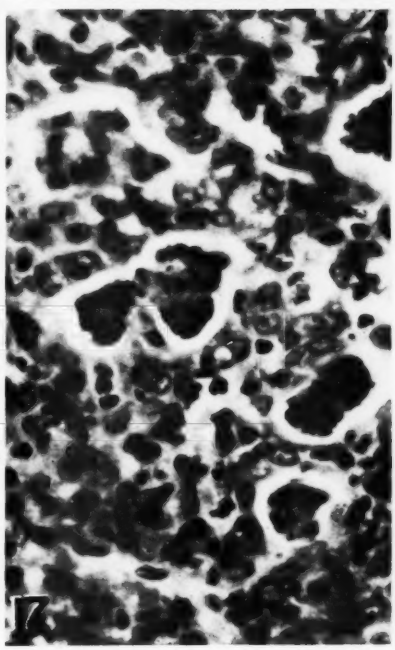
FIG. 17. Twenty-three days after inoculation. The lung has the general appearance of a gland. The alveoli are lined by cuboidal epithelium. The hyaline masses in the alveoli are surrounded by polymorphonuclear leukocytes. Hematoxylin and eosin stain. × 684.











Dubin

Mice Infected with Swine Influenza

PRIMARY SPLENIC NEOPLASMS *

WARREN L. BOSTICK, M.D.
(From the Division of Pathology, University of California Medical School, San Francisco 22, Calif.)

Splenic tumors were originally classified by Weichselbaum [1] in 1881 into three groups: spindle cell sarcoma, endothelial sarcoma, and lymphosarcoma. In 1923, Smith and Rusk [2] placed all primary splenic tumors as derivatives of the (a) capsule or trabeculae, *i.e.*, fibrosarcoma, (b) lymphoid tissue, and (c) vascular and sinus endothelium. Upon examining the literature, it immediately becomes apparent that these limited groups fail to include all of the types which have appeared in the 157 reported primary splenic tumors (42 since Howard's [3] report of 115 in 1929). Consequently, it becomes necessary to classify splenic tumors in accordance with all of the different cell types found in the spleen. From this it follows that there are seven fundamental neoplastic types, six of which have been reported. These seven are derived as follows: (1) vascular elements—angioma; (2) lymphoid tissue; (3) reticulo-endothelial cells—endothelioma and reticulum cell sarcoma; (4) embryonic inclusions—dermoids, epithelial cysts, and mesothelial inclusion cysts; (5) fibrous tissue derivative—fibrosarcoma and fibroma; (6) smooth muscle—leiomyoma; (7) nerve elements—neuroma or neurosarcoma.

Judged on a basis of 40 reported cases, the nonspecific diagnosis of splenic sarcoma is most frequent, and hemangioma next most frequent. The different types of reticulo-endothelioma and lymphosarcoma follow and are practically equal in number. Lymphangiomas are slightly less frequent, and the remaining types are relatively infrequent. As yet, neurosarcoma of the spleen has not been reported. Krumbhaar [4] reported that 0.64 per cent of all tumors of the body are primary in the spleen, and Lubarsch [5] found 3 angiomas in 19,000 autopsies. As to the splenic tumors which I am reporting in this article, two endothelial sarcomas, the reticulo-lymphosarcoma, the reticulum cell sarcoma, two angiomas, and the epidermoidal cyst were encountered in a series of 11,707 autopsies and 68,820 surgical specimens. Three cystic splenic tumors were encountered during this period, but these are not discussed on this occasion.

LYMPHOID TUMORS

The age distribution for the lymphoblastic tumors of the spleen ranged from 11 to 75 years in the reported cases. Most of the spleens weighed over 1000 gm. and were often nodular. In some cases these

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resembled a "sago spleen" and in others the nodules were up to 4 cm. in diameter. In the instances of rather diffuse nodularity, differences of opinion exist on very similar cases, as, for example, Ross' [6] diagnosis of lymphoid reticulosis of a nonneoplastic type which, in a very similar instance, was diagnosed by McNee [7] as lymphosarcoma. Frequently these patients suffer from an anemia of a secondary type, and, having also splenomegaly, are often placed clinically in the category of Banti's syndrome.

Microscopically, the essential cell type is the lymphocyte, which tends to appear somewhat immature, but may not be. Authors frequently refer to a slightly increased amount of cytoplasm over that which would be found in a lymphocyte in the blood. Mitotic figures are abundant, and the cells may lie on a reticular stroma but do not produce it. In the small-celled type giant cells are not found typically, but as the size of the cell increases to that of the large lymphocyte or lymphoblast, multinucleated forms are more likely to be found. Warren and Picena [8] proposed that when appreciable lymphopoiesis occurs the tumor should be called a reticulo-lymphosarcoma; when it is absent, a reticulum cell sarcoma.

Under lymphoid tumors in general, Hodgkin's granuloma can probably best be discussed. As a secondary tumor of the spleen it is quite common, but as a carefully proved primary lesion it is distinctly rare. Cases have been reported by Symmers,[9] Wade,[10] and Mellon.[11] Ewing [12] warned that many supposed Hodgkin's tumors will be found to belong correctly among other splenic neoplasms, probably most frequently the pleomorphic group of endotheliomata.

Tumors Derived from Reticulo-Endothelial Cells

primary reticulo-lymphosarcoma

Report of Case

Clinical Résumé. M. M. (no. CO-42-1313) was a white female, 90 years old. She had been in the hospital three times during the past 8 years, each time for degenerative vascular disease. Blood counts were always normal and lymph nodes were never enlarged. She last entered the hospital following a fall. A complete physical examination was done. No lymphadenopathy was observed, and other findings were only arteriosclerotic changes and bruises.

Laboratory examination showed the hemoglobin to be 70 per cent; red blood cells, 4.3 million; white blood cells, 14,000, with 87 per cent polymorphonuclear cells and 13 per cent lymphocytes. The patient expired. The clinical diagnosis was cerebral edema and arteriosclerotic heart disease.

A complete autopsy was done. The immediate cause of death was cerebral contusion. The spleen was four times normal size. It was diffusely adherent to the abdominal wall. On section it presented a

friable, congested parenchyma. Scattered throughout, but especially at its inferior pole, were nodules of gray, circumscribed abnormal tissue that were quite soft. They varied from 1.5 to 3.5 cm. in diameter, with the largest ones showing a confluence in the inferior pole. The abdominal cavity, liver, mediastinum, and lymph nodes were searched for other evidence of tumor formation, but none was found.

Microscopically, the tumor nodules in the spleen showed no tendency to be organoid and did not line sinuses, or form vascular channels (Fig. 1). In some areas degenerative changes were seen. The tumor cells were pleomorphic in size and type and occasionally multinucleated cells were present with oval or folded, dark nuclei. These cells had a moderate amount of cytoplasm. Also present, and comprising about 50 per cent of the cells, were round cells with indistinct outline and an oval or round nucleus without prominent nucleoli and rather uniform chromatin. Mitotic figures were quite frequent among these cells. They were about twice the size of a blood lymphocyte and resembled the basic type of that series. Finally, in the tumor there were seen scattered foci of cells possessing all of the characteristics of young lymphocytes. Search for eosinophilic leukocytes was fruitless, and special stains showed only an occasional larger tumor cell producing reticulum, although it was present in moderate amount in some areas.

Diagnosis. Primary reticulo-lymphosarcoma of the spleen.

Discussion. In view of the complete clinical study and the complete gross examination at autopsy, during which no evidence of tumor was found except that in the spleen, there remains no reasonable doubt that this is a primary splenic neoplasm. In its general pattern it is rather pleomorphic, with evidence of immaturity and with cellular proliferation of a reticular type. In addition, there is noted a significant number of round cells of the lymphocytic series and evidence of lymphopoiesis, which indicate a differentiation toward the lymphosarcoma type. With this tendency in mind, it is felt that this tumor is best placed in the group of reticulo-lymphosarcomas of the spleen.

RETICULUM CELL SARCOMA

Howard [3] and Gerundo and Miller [13] presented examples of primary reticulum cell sarcoma, and Gerundo and Miller considered that the reticulo-endothelial sarcoma reported by Langenstrass and Neumann [14] would have been best classified as a reticulum cell sarcoma. In establishing the diagnosis, the gross picture presents nothing characteristic, in that it may show a single mass in the form of a coarsely lobulated tumor, or exist as separate nodules. These nodules tend to be firmer than the surrounding spleen, and somewhat gray.

Microscopically, using the rather strict description by Oberling[15] and Warren and Picena,[8] the picture is as follows: The nuclei of the cells are irregularly distributed, oval or somewhat indented. These nuclei are quite prominent and have a well defined nuclear membrane enclosing a finely divided and scattered chromatin. In the more anaplastic forms one or two prominent nucleoli may be seen. The general paleness of the karyoplasm is often quite prominent. The cytoplasm in the undifferentiated forms consists of poorly outlined syncytial masses of undivided or slightly fenestrated protoplasm. In the more differentiated types the cell may be round, oval, or somewhat elongated, and 15 to 20 μ in diameter. The reticulum formation may vary from a few scattered, mostly intracytoplasmic, reticulum fibers to a quite complete network of reticulum passing between groups of individual cells. The cytoplasm may be rather abundant, and be either lightly acidophilic or basophilic. Occasionally multinucleated or binucleated cells are found, but these are not characteristic. Oberling stated that mitotic figures do not occur in great numbers, a conclusion quite the opposite to that of Parker and Jackson[16] and one not upheld in the reported cases. Lymphocytes may be seen in the stroma. The tumor cells should not show characteristics of tissue organization, such as actual new blood vessel formation. Some phagocytic activity of the cells may be demonstrable, but this is not considered essential for the diagnosis. In the most fully differentiated type of reticulum cell tumor, occasional areas of distinctly fusiform cells may be seen with some coalescence of the abundant reticulum fibers into strands of collagen. Such areas are not unlike fibrosarcomas, from which they may be clearly separated by examining the more undifferentiated portions of the tumor.

Gall and Mallory[17] have suggested the division of the reticulum cell sarcoma into the "stem cell" and "clasmatocytic" types. These appear to be valid subdivisions which had been recognized in the literature as variations in differentiation, but had not been individually named. This division, as applied to splenic sarcoma, needs further clarification.

Report of Case

L. A. (no. SP-44-1704) was a white housewife, 48 years of age. Her husband was living and well.

Present Illness. Five months before entry, the patient rather suddenly developed excessive malaise, dizziness, and nausea without vomiting. At that time she felt "feverish" and noted slight evening sweats. Her doctor found that her hemoglobin value was 40 per cent. He gave her iron, without effect. Two months before entry, while at rest, she suddenly developed a severe stabbing pain in the left upper quadrant which radiated to the left shoulder and was made worse with deep breathing and coughing. During 7 weeks of hospitalization, roentgenograms were made of the abdomen, and transfusions and supportive therapy were administered.

Numerous blood counts and blood cultures (all negative) and a sternal biopsy were done without establishing a specific diagnosis. While in the hospital she had daily afternoon fever of 100° F. and drenching night sweats. Petechiae were never noted. Weight loss was about 50 lbs.

Physical Examination. Ears, nose, and throat were negative on physical examination. Motion of the left half of the diaphragm was limited and the lower left pulmonary lobe showed dullness, decreased fremitus, and decreased breath and voice sounds. The heart was not enlarged, and the rhythm and rate were regular. P_2 was greater than A_2, and there was a loud, blowing systolic murmur over the precordium. Blood pressure was 110 mm. of Hg systolic, 165 diastolic. In the abdomen the upper left quadrant was tender and the spleen very large, extending to the umbilicus. The liver could not be felt, and no other masses or abnormalities were noted. Rectal and pelvic examinations were negative.

Laboratory Findings. Examination of the blood revealed the white blood cells to be 18,500, with 83 per cent polymorphonuclear leukocytes, 11 per cent lymphocytes, 5 per cent monocytes, and 1 per cent eosinophils. Red blood cells were normal and showed no sickle cells. Stains for malarial organisms were negative on four occasions. Hemoglobin, 65 per cent; red blood cells, 3.7 million. Cell volume, 34; color index, 0.74; volume index, 0.98; saturation index, 0.75; icteric index, 1.7; Rh factor, positive; red blood cell fragility, 0.48 to 0.36; Wassermann test, negative. Urine: Tests for albumin, Bence-Jones proteins, and sugar negative; occasional red blood cells and 1 white blood cell per high dry field. Serum protein total, 6.2 gm. per cent, with 3.6 gm. albumin and 2.2 gm. globulin. Agglutination tests for typhoid, paratyphoid, tularemia, undulant fever, and dysentery were negative. Sedimentation rate, 32 mm. (Wintrobe, noncorrected). Platelets, 390,000. Bleeding time (Ivy), 3 minutes. Coagulation time, 5½ minutes. Five blood cultures and a sternal culture were negative. Tuberculin tests at 1:1000 and 1:100, and a coccidioidin test were negative. Roentgenograms revealed an abdominal mass in the left upper quadrant; specific diagnosis not possible.

Course. The patient continued to have a daily fever from 38° to 39° C., with night sweats. Transfusions, sulfonamides, and penicillin were without effect. Splenectomy was decided upon, with a clinical diagnosis of probable tuberculoma of the spleen, or possible subacute bacterial endocarditis with old rheumatic heart disease and aortic insufficiency.

The spleen weighed 1450 gm. and measured 25 cm. in length. The surface was smooth and a dark slate-red. There were some irregularly shaped, pale areas on one surface. The hilar region revealed normal vessels and no neoplastic tissue. However, there were noted two small accessory spleens and four lymph nodes in this region. On section there was seen a large, central, yellowish white tumor mass (Fig. 2) occupying almost the entire organ and extending to within 3 to 4 cm. of each pole. The remaining splenic tissue existed as a rather compressed capsule 1 to 2 cm. thick. The tumor varied in consistency from a predominantly fibrous texture, through a softer sarcomatous consistency, into other areas of apparent mushy necrosis. A few small areas of cystic degeneration were noted which contained brownish fluid.

Microscopically, the predominant cell was one of moderate size with a rather abundant, pale, slightly granular cytoplasm having an indistinct cell membrane and containing a prominent, delicate, slightly

irregular oval nucleus. The nucleus had a distinct membrane, scanty chromatin, and one or several variably prominent nucleoli. Mitotic figures occasionally occurred. This rather succulent type of cell changed into a distinctly fusiform cell arranged in vague bands in some areas. In such areas reticular formation by the tumor cells was massive and collagen was evident. Elsewhere there was no collagen and, instead, the reticulum production by the cells was delicate and moderate in amount or completely absent. In some areas the tumor cells had phagocytized erythrocytes, granulocytes, or débris. There was no suggestion of formation of vascular channels by the tumor cells. In the background scattered lymphocytes and many eosinophilic leukocytes were noted. In many areas tissue degeneration with loss of all cellular detail was evident. The surrounding splenic tissue contained many red corpuscles, polymorphonuclear and lymphocytic leukocytes, and many eosinophilic granulocytes. The germinal follicles were of fairly normal appearance and the capsule was negative (Figures 3 to 5).

The sections of the hilar lymph nodes and accessory spleens showed no neoplastic tissue and a pattern that was normal.

Diagnosis. Primary reticulum cell sarcoma of the spleen.

Discussion. The moderate-sized, often rather syncytial-appearing cells, showing delicate reticulum production and scattered evidence of phagocytosis, point distinctly toward the reticulum cell origin of this tumor. In certain areas this tumor becomes highly "differentiated" (Oberling [15]) and assumes a fusiform cellular pattern together with heavy reticulum production and collagen. In such areas the cells have differentiated toward fibroblasts and the tumor is not unlike a fibrosarcoma. The term reticulo-fibrosarcoma has been suggested for such a tumor. Perhaps as a descriptive phrase this may be justified. However, such a term is not established in the literature and certainly Oberling's class of "differentiated reticulum cell sarcoma" more nearly expresses the correct cytological derivation of these tumors.

ENDOTHELIAL SARCOMAS

In the literature, splenic endotheliomas are next to, or equal to, lymphosarcomas in frequency. In six carefully studied cases obtained from different reports in the literature, the ages ranged from 33 to 67 (average, 52 years), the incidence of male to female was equal, and the spleens weighed from 420 to 5300 gm. with an average of 1700 gm. Although it is one of the more frequent tumors of the spleen, Ewing [12] remarked that in the spleen, as in lymphoid tissue in general, endothelial cells tend to lack many of their classical characteristics and that tumors derived from them are considered subvarieties of sarcoma.

For that reason a diagnosis of endothelioma should be carefully established, and this group must not serve as a ragbag for poorly defined tumors.

Connor,[18] in his study of marrow tumors of the endothelioma group, formulated three fundamental types which correspond well with similar tumors found elsewhere in the body. The first type is the angio-endothelioma, characterized by the differentiation toward vascular tissue with formation of sinuses and vascular spaces. The second type is the diffuse tumor in which the cells appear in sheets and masses, forming no definite structure, but sometimes, and in some areas, the cells are in rows or small alveoli. The third group is designated the reticular type in that its cells are united by fine protoplasmic processes and there is a variable amount of reticulum production. This form is usually regarded as a more primitive type, intermediate between the first two forms. This general classification applies very well to the endothelial tumors of the spleen and lymphoid tissue in general. It follows, therefore, that the establishment of the identity of a mesenchymal tumor of the spleen would be greatly aided by the discovery of the formation of sinuses, alveolar spaces, or a radiating perivascular arrangement.

Specific cytological details of the endothelioma of the spleen have not been established in the literature, probably because of the rather wide variation which is found. However, a composite picture of the cell type as found in a series of well authenticated cases should serve as a base line for classification. The cell tends to be rather large, some 20 to 30 μ in diameter. Its outline, in areas showing little or no structural differentiation in the direction of lining tissue spaces, is usually round or oval, and in more fibrous-appearing areas may be elongated. The cytoplasm is rather pale or lightly acidophilic, and tends to be not very abundant. The nuclei are prominent, usually oval, less frequently lobulated, and with a distinct nuclear membrane. A finely reticulated karyoplasm contains some dark chromatin particles and usually one or two eccentrically placed, dark nucleoli. Multinucleated giant cells in moderate or occasionally fairly large numbers are usually found, and mitotic figures are characteristically frequent. Phagocytosis by the tumor cells may be seen, but this does not differentiate them from reticulum cells or mesenchymal cells in general. It will be noted that in the description of the nucleus there is a marked difference from the typical endothelial cell. In the exceptional tumor, the delicate, non-nucleolated classical type of endothelial cell will be found. Ewing [12] recognized this difference in endothelioma of the lymphoid system.

From this cytological description, it can be appreciated that the real problem in the classification of splenic tumors lies between the

reticulum cell sarcoma and the diffuse endothelial sarcomas producing reticulum (reticulo-endothelial sarcomas). Unless one wishes to accept the rather strict criteria for reticulum cell sarcoma, as proposed by Oberling [15] and Warren and Picena,[8] this separation probably cannot be made consistently.

PRIMARY ENDOTHELIOMA

Report of Cases

Clinical Résumé. C. W. (no. SS-41-1066) was a white switchman, 57 years old. He was entirely well until 2 weeks before entry, at which time he began to notice crampy, left-sided pain after eating, some nausea, but no vomiting. There had been no weight loss. Physical examination revealed no abnormalities except those referable to the abdomen. This presented generalized tenderness and rebound tenderness in the left lower quadrant. The general picture was that of an early, generalized peritonitis.

Examination of the blood showed: hemoglobin, 80 per cent; red blood cells, 3.8 millions per cmm.; white blood cells, 24,000 per cmm. with 90 per cent polymorphonuclear leukocytes; platelet count, 150,000 per cmm.; clotting time, 10 minutes, bleeding time, 2.5 minutes; Wassermann test, negative, nonprotein nitrogen, 27 mg. per cent.

At operation, a considerable amount of free blood was seen in the peritoneal cavity, and the spleen was found to be torn at one pole. The spleen was removed. No other abnormalities were encountered. The patient made an uneventful recovery, and further study revealed no evidence of a lymphomatous tumor in the lymph nodes.

The spleen weighed 455 gm. The external appearance was normal except for a rupture at one pole. This was in the form of a hole that measured 1 by 1.5 cm. Upon section, it was seen that this rupture had occurred in a tumor measuring 3 by 4 cm. in cross section. This tumor was pink-white, soft to palpation, and with no gross evidence of necrosis except at the point of rupture. The tumor was quite distinct from the rest of the spleen and there were a few small satellite tumors immediately surrounding the main mass. A small accessory spleen also was removed.

Microscopical examination showed a capsule which, where it was not broken, was well formed. The splenic tissue revealed compression and thickening of the trabeculae, but congestion was not prominent and the malpighian corpuscles were normal. The neoplastic tissue was clearly separated from the surrounding splenic tissue. The tumor pattern was somewhat distorted by the presence of an inflammatory reaction. Nonetheless, there was a distinct picture of tumor cells lying attached to each side of delicate, irregular, but roughly parallel connective tissue septa, and projecting into the intervening spaces (Fig. 6). In some areas this property of lining tissue spaces was more prominent than in others. The neoplastic cells revealed some pleomorphism, and were of moderate size, being from 21 to 28 μ in

diameter. The cytoplasmic outline was indistinct, usually rather irregular but occasionally round. The cytoplasm was pale and not abundant. The nuclei were oval or rounded and occasionally slightly folded. The nuclear membrane was distinct and moderately heavy. The karyoplasm was rather pale and contained scattered and somewhat coarse chromatin with usually one rather prominent, slightly eosinophilic nucleolus. A moderate number of multinucleated tumor cells were seen, and mitotic figures and pyknotic nuclei were frequent. In many areas there was a diffuse polymorphonuclear leukocytic infiltration which was more abundant in and near areas of hemorrhagic degeneration. Associated with this was a scanty but evident eosinophilic leukocytic infiltration. Lymphocytes were not seen except where left behind by an invaded splenic pulp. Silver stains showed throughout an increased amount of reticulum, which was intercellular in distribution and which was not seen to arise within the tumor cells.

Diagnosis. Primary endothelioma of the spleen, with focal necrosis and rupture.

Discussion. The inflammatory reaction somewhat confuses the microscopical appearance of this tumor because of the distinct infiltration of eosinophils, which superficially suggests a Hodgkin's type of reaction. However, it is felt that this is purely an inflammatory reaction. In addition the tumor pattern lacks the background of fibrosis and varying cellular types found in Hodgkin's disease. The formation and lining of tissue spaces by the tumor cells is distinct, and somewhat resembles the lining of the splenic sinusoids, in that the tumor cells lie on either side of delicate connective tissue strands. With their moderate neoplastic pleomorphism, these cells are in distinct contrast with the cells found in the primary splenic endothelioma of the case described below. In the latter the tumor cells more closely resembled the standard accepted type for endothelial cells. Such is usually not the case, for the primary splenic endotheliomata more frequently present the pleomorphic pattern of the tumor presented by this patient.

Clinical Résumé. E. J. (no. SS-41-1100) was a white housewife, 55 years old. Ten years before her present hospitalization a mass was noted in the abdomen, and after careful clinical investigation was diagnosed as most probably a cyst of the kidney. At operation a large cyst was found behind the stomach. This arose from the region of the tail of the pancreas and was retroperitoneal. The spleen and kidneys appeared normal to inspection and palpation. The cyst was evacuated and a specimen was taken for biopsy. The pathological diagnosis was fibrous tissue without epithelium and showing chronic inflammation. The surgical diagnosis was pancreatic cyst.

The present entry into a hospital was occasioned by the onset of convulsive seizures or "spells" which, even after careful investigation, were not identified as to etiology. In the hospital these increased gradually in frequency in spite of therapy, and the patient went progressively downhill to her death.

Physical examination revealed no abdominal masses, although urograms showed some calcification in the region of the spleen or pancreas. Laboratory reports showed a drop in the hemoglobin from 82 to 50 per cent, with a corresponding drop in red blood cells from 5.0 to 3.2 million. White blood cells dropped from 12,000 to 7,000, with 70 per cent polymorphonuclear leukocytes, 20 per cent lymphocytes, 2 per cent eosinophils, 4 per cent monocytes, and 1 per cent basophils. The blood smear was normal, and platelets were numerous. The Wassermann test was negative and the glucose tolerance curve normal. The serum calcium was 8.3 mg. per cent, and nonprotein nitrogen, 32 mg. per cent.

A complete autopsy was done, and the essential findings were bronchopneumonia and a tumor of the spleen. There was no evidence of metastases. The spleen weighed 1340 gm. and measured 19 by 16 by 13 cm. One pole was occupied by a nearly round tumor mass, 12 cm. in diameter. The capsule of the tumor was quite dense and in some places contained calcified material. In the center of the tumor, degeneration was seen.

On microscopical examination (Figs. 7 and 8) the splenic tissue showed a normal capsular and trabecular pattern. Splenic sinusoids and pulp tissue contained no abnormal cellular types and lacked unusual congestion. The malpighian bodies were discrete and of moderate size. The arterioles showed thickened walls, but no perivascular fibrosis. In some sections the splenic tissue ended abruptly at points of neoplastic invasion. The tumor had invaded the spleen and also the muscles of the abdominal wall where the tumor had become adherent to the wall.

The essential pattern of the tumor was one of a rather delicately fenestrated mass of relatively uniform cells, which might assume an alveolar pattern or which might be arranged along, and line, distinct clefts in the tumor. This general pattern might be distorted by areas of degeneration, containing calcium salts and cholesterol crystals, with loss of cellular structure except around larger vessels, where a peritheliomatous arrangement of the neoplastic tissue was striking.

The tumor cells were fairly uniform in type and size. The majority were round or oval, but might be elongated in areas of distortion or degeneration. The cytoplasm was lightly acidophilic, only moderate in amount, very poorly outlined, and frequently flowed imperceptibly into adjacent stellate tumor cells. This syncytial appearance was frequent, but giant cells were not seen. The nuclei were usually 10 to 14 μ in diameter, and oval. The nuclear membrane was distinct, but not coarse, and enclosed a gray nucleoplasm of ground-glass appearance containing chromatin dust and practically never any prominent nucleoli. Mitotic figures were rather infrequent. Silver stains showed only a very rare tumor cell producing reticulum. In areas of old

hemorrhage the neoplastic cells had occasionally phagocytized some iron pigment.

Diagnosis. Primary endothelioma of the spleen.

Discussion. This tumor presents no problem in classification, in that it shows certain differentiating characteristics. It obviously arose from the spleen, and the opportunity to observe the spleen 10 years before is rather unusual. The growth energy of the tumor was of a rather low order as evidenced by calcification and infrequent mitotic figures. The distinctly endothelial characteristics of the tumor cells are infrequently encountered in an endothelioma of the spleen, in which more bizarre patterns are common.

Benign Lymphangiomas and Hemangiomas

The reported incidence of benign lymphangiomas and hemangiomas varies rather widely, in that Lubarsch [5] found only 3 cases in 19,000 autopsies, yet Schottenfeld and Wolfson [19] reported an incidence of 0.14 per cent in 2,800 autopsies and Pines and Rabinovitch [20] 0.16 per cent in 3,676 consecutive autopsies. These latter authors [20] found 36 reported examples of true benign hemangioma in the literature up to 1941 and added 6 more cases of their own. The 2 reported in this paper bring the total to 44. In 1938, Akcakoyunlu [21] was able to find only 21 acceptable tumors of this type.

The average age of the patients with these tumors is from 35 to 45 years, with extremes of 4 months to 72 years. The incidence in males and females is about equal. The largest tumor reported weighed 7240 gm. (Akcakoyunlu [21]). The smaller ones are usually found incidentally at autopsy and are entirely silent clinically. Those surgically removed have usually caused symptoms and are larger. In 16 cases involving rather large tumors, Schottenfeld and Wolfson [19] listed findings as follows: abdominal tumor, 100 per cent of cases; pain, 62 per cent; anemia, 12 per cent; ascites, 12 per cent; and weight loss, 18 per cent. Except for anemia in some cases, there are no characteristic changes in the hemogram.

It is probable that the majority of these tumors arise on the basis of a congenital nevus, and slowly enlarge over a period of years. Active proliferation is difficult to establish except in the more aggressive examples. Usually physical factors dependent on congestion and hemorrhage play a prominent part, and occasionally involution may occur through thrombosis and fibrosis.

In classification, these tumors readily fall into the standard nomenclature of angiomas in general, including capillary, cavernous, telangi-

ectoides (or cystic), and mixed types, depending on the average size and dilatation of the tumor spaces. It must be emphasized that the predominant feature in these tumors is the formation of vascular channels, and not the cells involved in their formation. When it is evident that the main element consists of masses of cells which line undifferentiated vascular spaces, the tumor is essentially an endothelioma, and is properly designated a hemangio-endothelioma.

HEMANGIOMA

Report of Case

J. G. (no. A-24-205) was a Mexican, 21 years old. A splenic tumor was found incidentally during a routine autopsy, the patient having died of subacute disseminated pulmonary tuberculosis.

On gross inspection, the spleen was slightly increased in size, had a moderately firm pulp appearing to be only slightly congested. There was no gross evidence of tuberculous involvement. On section there was noted a rather soft peripherally placed area, measuring 2 cm., which was somewhat raised and darker than the rest of the tissue. It was seen to consist of several, small, cystic cavities filled with blood in an area which was not infarcted. It was interpreted as a hemangioma grossly. Microscopically, it was found to be formed of simple blood-filled spaces lined by flat endothelial cells.

Diagnosis. Hemangioma of the spleen (cavernous type).

LYMPHANGIOMA

Report of Case

C. M. (no. SA-35-166) was a white male, 35 years old. A splenic tumor was found incidentally during a routine autopsy, the patient having died of a ruptured luetic aortic aneurysm.

The spleen was of normal size and of firm consistency with a reddish purple color. The malpighian corpuscles were easily seen. At one pole there were seen a few, small, cystic structures each measuring 2 to 3 mm. in diameter.

Microscopically, the cystic structures were seen to be of variable size (Fig. 9) and to be filled with a pink, serous-appearing fluid containing no erythrocytes. Some spaces were fused with broken septa floating in their common lumen. They were lined with a single layer of low cuboidal, inactive endothelium beneath which was a thin connective tissue layer abutting directly on splenic tissue. The splenic pulp throughout was rather congested, but otherwise this tissue was normal. The arterioles showed no fibrosis. They were delicate with only slight intimal thickening.

Diagnosis. Lymphangioma of the spleen (cavernous type).

Fibromas and Fibrosarcomas

It was the fibrosarcoma which Weichselbaum [1] described in 1881 that prompted him to add spindle cell sarcoma to his other two basic types of sarcoma of the spleen: the lymphosarcoma and the endotheliosarcoma. Since his report only two other examples of this tumor have been reported, one by Jepson and Albert [22] and the other by Heinricius.[23] They are similar microscopically to fibrosarcoma found elsewhere in the body, and consist of spindle cells predominantly arranged in fasciculi and sheets with only occasional oval-shaped tumor cells. Production of intercellular collagenous material is noted in the more differentiated areas, and the formation of vascular channels by the tumor is not seen. The occasional well differentiated reticulum cell sarcoma, which in a few areas may be spindle-celled and produce dense reticulum together with some collagen, must not be confused with the fibrosarcoma. The resemblance between the two is only superficial and by examining the more undifferentiated areas the identity of the separate cell types is apparent. Heinricius' case showed myxomatous degeneration in some areas.

Although Krumbhaar [4] stated that simple fibromas are found in the spleen, as they doubtlessly are, no example of a reported case could be found in the literature, which fact probably speaks more for their insignificance than their rarity.

Leiomyosarcoma

The single reported example of a primary leiomyosarcoma of the spleen in any animal occurred in a bovine, and was described by Feldman.[24] It had all of the characteristics of this neoplasm as it occurs elsewhere in the body, and was carefully studied by differential stains.

Dermoid and Epidermoid Cysts

Weil, Roux-Berger, and Scemama [25] have pointed out the two possible derivations of dermoid and epidermoid cysts of the spleen. The dermoid tumors and the distinctively epithelial cysts are satisfactorily explained on the basis of congenital epithelial inclusion. Other less distinctively epithelial linings probably represent metaplasia of mesothelial or endothelial-lined spaces. These authors used the term "epithelial cysts" rather loosely, including those which are apparently only metaplastic in origin. Their example possessed no distinctive epithelial characteristics other than being lined with several layers of simple squamous cells in a few areas. They accepted as epithelial cysts those with practically no cellular lining, emphasizing the apparent absence of epithelial lining cells in the obviously epidermoid cyst

of the spleen reported by Kumaris [26] which contained hair and "dermoid balls."

Keratinization, hair, and epidermal glands constitute conclusive evidence. Lacking these, intercellular bridges, pigmentation, and multiplicity of cell layers are highly suggestive, if at least two of them are present. In tumors containing multiple cysts in which only one or two of the cysts appear epithelial, the rest being structurally mesodermal, probably the apparent epithelium is due to mesodermal metaplasia. Herein lies the importance of observing the above-described criteria. In the absence of keratinization, hair, or epidermal glands, simple metaplasia cannot be positively ruled out. From the behavior of the lining cells of the female genital system it is obvious that mesodermal derivatives possess the property of forming a morphologically typical endodermal or ectodermal epithelium. This may partly account for Custer's [27] conclusion that epidermoid cysts of the spleen are not rare in that he encountered 5 cases in 5,000 autopsies. Every effort should be made to establish the origin of the lining cells of each splenic cyst under consideration.

In the literature, definitely epidermoid splenic cysts were reported by Andral [28] and by Kumaris,[26] in that they contained hair and keratin. Velasco Suarez and Angel Etcheverry [29] reported a dermoid cyst of the splenic hilum (not in the spleen) associated with hemolytic icterus and anemia. Other authors have reported splenic cysts as epidermoid in origin on the basis of squamous epithelium, at points multilayered, and almost always having intercellular bridges. Such reports were made by Weil, Roux-Berger, and Scemama,[25] Carnett, Bates, and Linney,[30] Shawan,[31] Lereboullet, Grégoire, Bernard, and Ibarran,[32] Dinand,[33] and by Montgomery, McEnery, and Frank.[34] These last authors reported 2 cases. The case presented in this paper brings the total number of reported cases of apparently epithelial-derived tumors and cysts of the spleen to 11.

All of the primary carcinomas of the spleen in the literature were reported during an era when there was still technical and morphological confusion between certain types of carcinoma and sarcoma. Therefore they were all probably sarcomas, erroneously diagnosed as primary carcinoma. However, there is always the possibility that one of these may have developed from an aberrant epithelial remnant. No such example could be found in the literature.

EPIDERMOID CYST

Report of Case

D. R. (no. SP-38-1340) was a white school girl, 14 years old. She had noticed a swelling in the left upper quadrant for the past 1½ years, which at first gave no symptoms. Later, and at intervals of several months, she would experience severe

localized abdominal cramping pains, that were unassociated with clinical signs and which would subside within a week. Physical examination was negative except for the presence of a large, rather hard, smooth mass in the region of the spleen. It was not fixed; it extended to the midline, and was 4 fingersbreadth below the left costal margin.

Laboratory work showed a normal phenolsulfonphthalein excretion and Addis count. Examination of the blood showed the hemoglobin to be 83 per cent, with 4.0 million red blood cells. White blood cells were 8,800, with 71 per cent polymorphonuclear leukocytes, 23 per cent lymphocytes, 4 per cent eosinophils, and 2 per cent monocytes.

The clinical diagnosis was probable renal tumor, and an exploratory laparotomy was performed during which a large spleen was removed. The patient made an uneventful recovery.

The spleen measured 20 by 12 by 7 cm. The greater part, including the upper pole, consisted of a cystic cavity (Fig. 10) which measured 10 cm. in diameter. With the cyst empty, the spleen weighed 380 gm. The cyst fluid was dark red-brown; microscopically it contained a few red blood cells, many granular leukocytes, and a few pigment-containing macrophages. The cystic wall varied in thickness, averaging 0.5 cm. Its inner surface was heavily and coarsely trabeculated by thick, pale bands of tissue. The splenic pulp appeared fairly normal but rather fibrous.

Microscopical sections of the wall of the cyst showed an irregular impocketing of a lining of stratified squamous epithelium (Fig. 11). This covered the whole interior, and there were no areas suggestive of a metaplastic process. The epithelium varied in thickness from several layers to a dozen or more. Some of the surface cells were hydropic, but no distinct keratinization had occurred. Many cells just beneath the surface showed the characteristics of the stratum granulosum. Deeper, the typical prickle cells of the stratum spinosum were found. This epithelium stained yellow with van Gieson's stain. It rested on a dermal layer composed of rather heavy connective tissue which merged imperceptibly into the splenic tissue. Away from the wall of the cyst the splenic tissue was normal in all respects, but became more fibrous as the wall was approached.

Diagnosis. Epidermoid cyst of the spleen.

Summary

A classification of primary splenic tumors based upon histogenesis is presented. Arranged in their order of frequency, as found in the literature, these groups are as follows: (1) vascular—lymphangioma and hemangioma; (2) lymphoid—lymphoma; (3) reticulo-endothelial cells—endothelioma and reticulum cell sarcoma; (4) embryonic inclusions—epithelial cysts, dermoids, and mesothelial inclusion cysts; (5) fibrous tissue—fibrosarcoma; (6) smooth muscle—leiomyosarcoma; (7) nerves—neurosarcoma. No example of a neurosarcoma of the spleen has been found in the literature.

Case reports are presented of: (1) primary reticulo-lymphosarcoma of the spleen in a woman, 90 years old; (2) a primary reticulum cell sarcoma of the spleen; (3) primary splenic endothelioma, two examples; (4) one cavernous hemangioma and one cavernous lymphangioma; (5) epidermoid cyst of the spleen, the eleventh reported in the literature.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

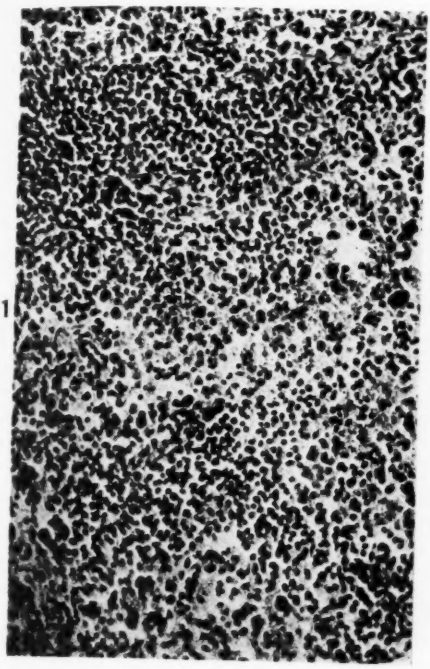
PLATE 201

FIG. 1. (Case CO-42-1313.) Primary splenic reticulo-lymphosarcoma. Large cells of reticulum type can be identified, intermingled with more numerous typical lymphocytes. Mitotic figures are moderately frequent, eosinophilic leukocytes absent. × 170.

FIG. 2. (Case SP-44-1704.) Primary reticulum cell sarcoma of the spleen. Total splenic weight, 1450 gm. The tumor was almost solitary, with only a few, small, satellite nodules. In many areas it was soft and necrotic and in other areas the tissue was rather dense and fibrous. The cut surface of the tumor at the plane sectioned measured 6 by 9 cm.

FIG. 3. (Case SP-44-1704.) Reticulum cell sarcoma of the spleen. The production of delicate reticulum by tumor cells is seen, as well as their indistinct cytoplasmic outline, pale appearance, and vesicular nuclei. × 170.

FIG. 4. (Case SP-44-1704.) Primary reticulum cell sarcoma of the spleen showing an area of extensive differentiation into a fusiform cellular type having abundant reticulum and some collagen production (collagen is black). × 170.



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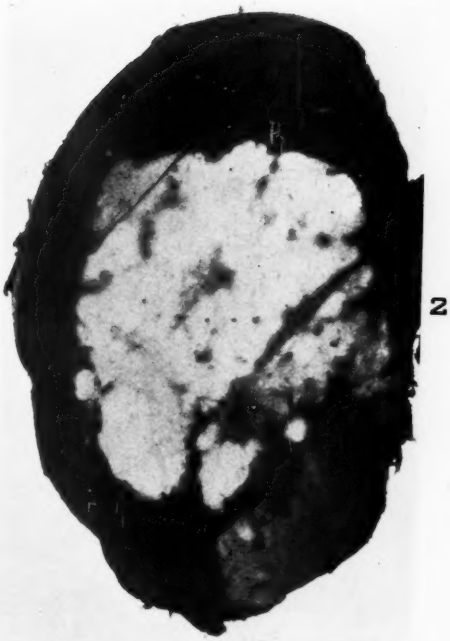

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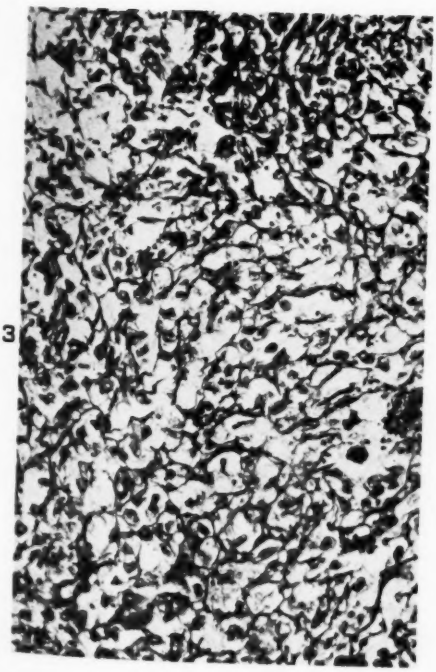

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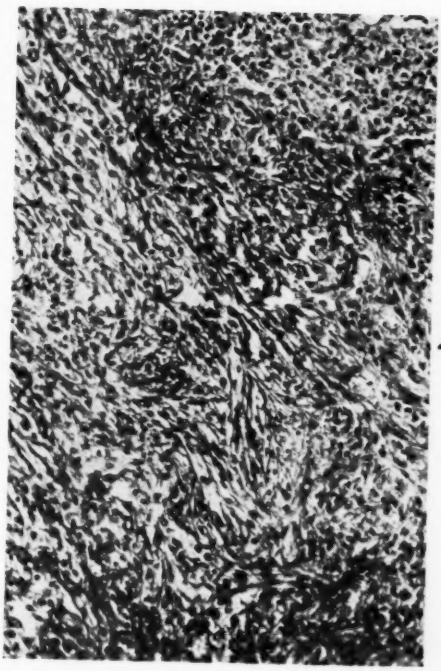


4

Bostick

Primary Splenic Neoplasms

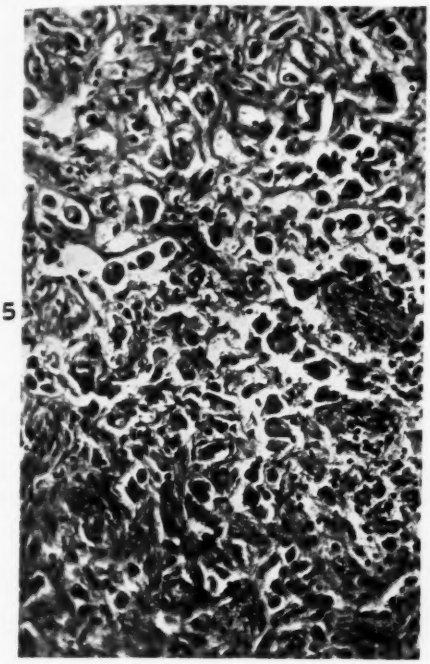
PLATE 202

FIG. 5. (Case SP-44-1704.) Primary reticulum cell sarcoma of the spleen showing actively phagocytic tumor cells containing erythrocytes and débris. × 200.

FIG. 6. (Case SS-41-1066.) A field from the primary endothelioma of the spleen, showing a moderate degree of cellular pleomorphism with cells of a rather vesicular, moderate-sized type. Mitotic figures are evident as is the distinct tendency for the neoplastic cells to lie against a delicate fibrous stroma and to line tissue spaces. × 170.

FIG. 7. (Case SS-41-100.) Primary endothelioma of the spleen. The cell type is uniform and cellular outline indistinct. A tendency to line spaces is evident. Mitotic figures are rare. × 170.

FIG. 8. A higher magnification of the field shown in Figure 7. Mitotic figures are not seen and the somewhat syncytial aspect of the cytoplasm is evident. The nuclei have very fine chromatin material and no apparent nucleoli. × 270.



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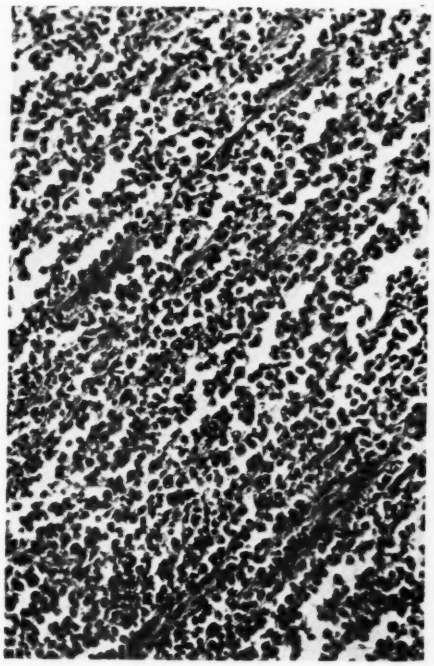

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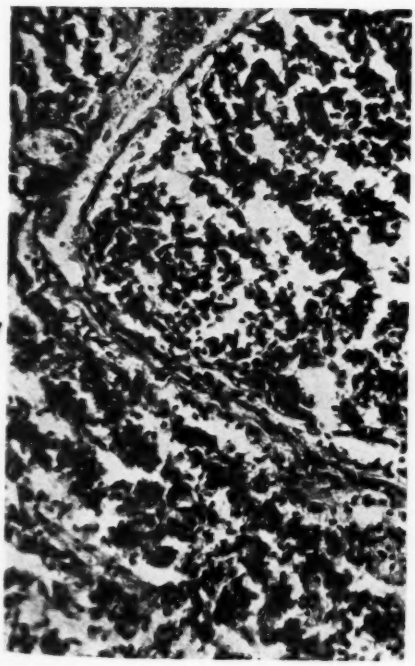

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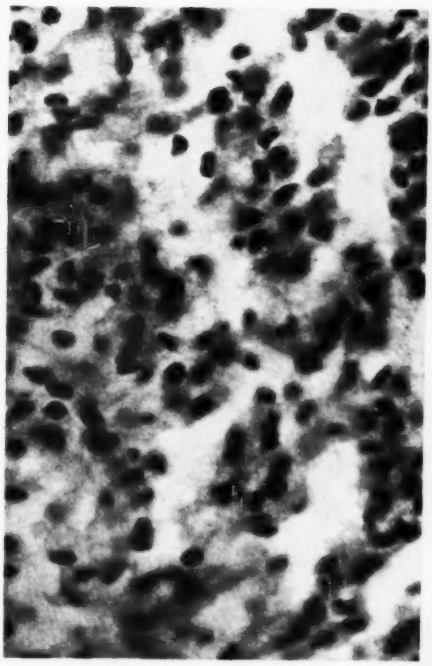

8

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Primary Splenic Neoplasms

PLATE 203

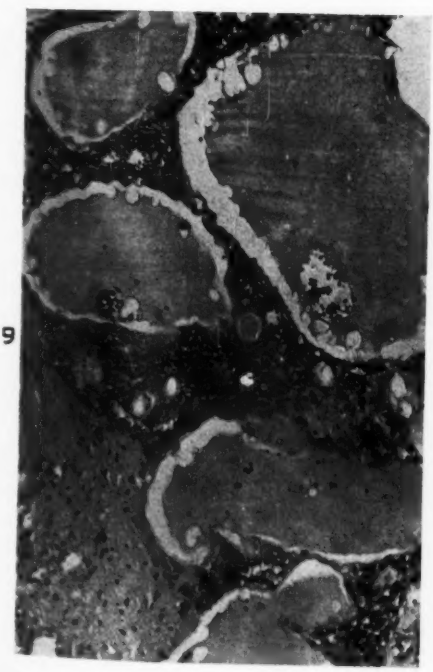
FIG. 9. (Case SA-35-166.) Cavernous lymphangioma of the spleen. The vascular spaces are filled with light eosinophilic amorphous serum containing a few leukocytes. The lining endothelium is flat and inactive. ×170.

FIG. 10. (Case SP-38-1340.) An epidermoid cyst of the spleen weighing 380 gm. when empty and revealing a single large cavity with coarsely trabeculated wall.

FIG. 11. (Case SP-38-1340.) Photomicrograph of the lining of the epidermoid cyst shown in Figure 10. It consists of polygonal multilayered squamous epithelium. Intercellular bridges are present as well as a stratum granulosum but there is no keratin. × 170.

10

9



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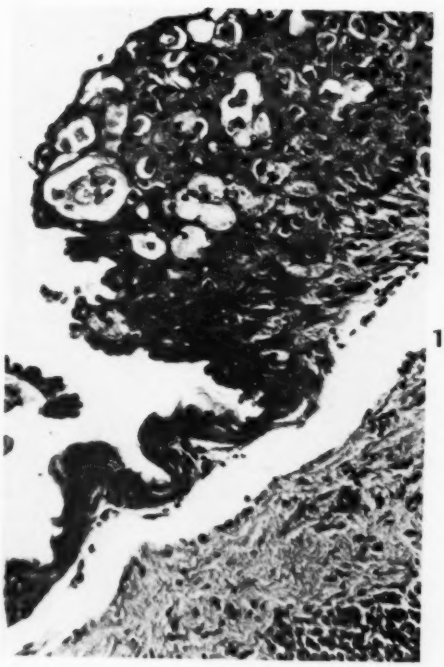

Bostick

Primary Splenic Neoplasms

GIANT CYSTIC ARRHENOBLASTOMA OF THE OVARY CONTAINING ENTODERMAL EPITHELIUM AND A CARCINOID *

PHILIP H. HARTZ, M.D.
(From the Public Health Service, Curaçao, N.W.I.)

Although, since Meyer's [1] fundamental studies, the arrhenoblastomas have been generally accepted as a special group of tumors to be distinguished from other ovarian neoplasms, their origin and nature are still being discussed. Several authors believe that these tumors represent teratomas containing virilizing elements. Kanter and Klawans [2] reported an arrhenoblastoma which contained young, partially developed cartilage, and Krock and Wolferman,[3] analyzing the 70 cases available in the literature, found suggestions of tridermal elements in 24 cases. Other authors, *e.g.*, Geist,[4] considered the teratomatous origin of the arrhenoblastomas as extremely unlikely. Rhoden [5] believed that the difficulty in deciding between a teratomatous and nonteratomatous origin can be avoided if it is kept in mind that in both cases the tumor originates from the sex-cell. However, for this theory no wholly convincing proof has been offered. The case to be reported has three distinguishing features: (1) the enormous size of the tumor; (2) its great pleomorphism; (3) the presence of epithelial formations which could be identified with certainty as entodermal elements.

REPORT OF CASE

A colored girl, native of Aruba, N.W.I., and 20 years old, was sent to the hospital with the following history. Menstruation had started at the age of 16; the flow had always been very scanty and usually consisted only of some "spotting." Fifteen months before, menstruation had stopped completely. She consulted a physician, who on general examination found pronounced hirsutism, atrophy of the breasts, and absence of the normal womanly contours. Gynecological examination revealed a hypertrophic clitoris, about 3½ cm. long, and a pelvic tumor, which could be felt 4 cm. above the symphysis. Operation was refused and several other physicians were consulted. In the meantime the abdomen of the patient enlarged steadily until it surpassed the size of a full-term pregnancy, whereas the general appearance of the patient had become like that of a man. Permission for operation was obtained at last and in view of her general condition laparotomy was performed on the day of her admission into the hospital. An enormous tumor of the right ovary was found. There were no adhesions with the intestines or with the abdominal wall. The uterus and the left ovary showed no pathological changes. The tumor was removed without difficulty (Dr. M. J. Hugenholtz). The postoperative course was uneventful and the patient returned to Aruba after 2 weeks. She was re-examined 3 weeks after the operation, but no change was noted. Eight months after the operation the patient was seen again. She had changed completely and, in the words of her physician, had become a pretty girl. The superfluous hair had fallen out and she had gained weight. Menstruation had occurred 2 months after operation and had been recurring regularly, the flow lasting 5 days.†

* Received for publication, December 4, 1944.

† I am indebted to Drs. J. Oduber and W. de la Fuente for part of this information.

Gross Findings

The specimen consisted of a large, cystic tumor, weighing 12.1 kg. The surface was smooth, the color was bluish red. The tumor contained a slightly turbid, brown-red fluid. The thickness of the cyst wall varied between 4 mm. and 3 cm. The inner lining of the cyst was pale red and slightly irregular; sometimes it resembled hypertrophic endometrium. The thickness of this pale red tissue varied. In several places islands of this tissue were completely enclosed by grayish, edematous connective tissue, which made up the rest of the cyst wall. Several small hemorrhages were noted.

Microscopical Examination

Thin blocks of tissue were fixed in the Bouin-sublimate mixture and embedded in "tissuemat" after treatment with methyl benzoate celloidin. The sections were stained with hematoxylin and azophloxine, hematoxylin and mucicarmine, Petersen's [5a] acid alizarin blue-aniline blue, the azan stain, Masson's tetrachrome, 1 per cent aqueous safranine, phosphotungstic acid hematoxylin in the modification of Masson,[6] and Masson's stain for argentaffine cells.[7]

The wall of the cyst consisted of the neoplastic tissue *sensu strictiori* and of connective tissue; both were present in varying quantities. Sometimes the neoplastic tissue occupied the greater part of the cyst wall but in other places only thin strands of it were found. The tumor consisted of different kinds and types of cells and tissues which very often mingled or merged into each other. In many places the dominating cell type was a relatively small cell with a dark-staining nucleus (Fig. 1). The nuclei of these cells were oval and their longest diameter measured 8 to 12 μ. They contained one or two small nucleoli. There was but little protoplasm, which stained lightly. Since in most places the cells were closely packed, it was very difficult or impossible to distinguish the cell borders. The cells were arranged in smaller or larger groups or alveoli (Fig. 2) which were surrounded by thin septa, carrying thin-walled blood vessels. With the connective tissue stains, very thin, often branching fibers were found inside the cell groups; in many places they surrounded individual cells. It could not be determined with certainty whether the fibers were intraprotoplasmatic. Sometimes the cells merged imperceptibly into small, short, spindle cells. Also groups of cells with paler nuclei were found; intermediate forms between the cell types mentioned were frequent.

In and between the nests of small cells, cells of another, larger type were found. These cells occurred as isolated specimens, in small groups or arranged in cords. These groups and cords were always well de-

limited by connective tissue, resembling a basement membrane. The cells had a distinct cell body with a coarsely vacuolated and reticulated protoplasm; the size of the cells and nuclei varied between wide limits; sometimes giant cells with nuclei measuring 40 μ or more were found (Fig. 3). Density of the chromatin varied. In most nuclei there was a small nucleolus. Intermediate forms between the small and large cells were present. The cords of large cells showed a tendency to form lumina so that more or less regular tubules appeared, lying between the groups of small dark-staining cells. The cells lining the tubules showed, in general, the same reticulated protoplasm, and assumed a prismatic form. In many places terminal bars were found. Sometimes the apical protoplasm of the cells contained very small, closely packed granules. No connective tissue fibers were found inside the basement membrane of these cords and tubules. In other fields the cell cords were broader; the cells had light-staining, band-like nuclei and the same reticular protoplasm (Fig. 4). They were arranged in two to four rows perpendicular to the basement membrane. In the different cell types only a few mitotic figures were found.

At the periphery of, and sometimes also inside, the cellular neoplastic tissue there were larger cells of a different type. They had round to oval nuclei with one, sometimes two, conspicuous nucleoli and a granular protoplasm, which stained dark red with hematoxylin-azophloxine and with Masson's tetrachrome, and dark blue with phosphotungstic acid hematoxylin. The protoplasm sometimes contained brown pigment granules, which stained black with silver. In many cells there were small vacuoles. The cells were lying singly or in small groups or rows. When lying in close contact with each other, often a small cleft, completely surrounded by protoplasm, was observed, producing a certain resemblance to liver cells and bile canaliculi (Fig. 5). These cells were also found in combination with another type of tissue, which will be described later.

Apart from the tubules which were found between the cells of the small type, other tubules were found lying in groups in the connective tissue of the cyst wall. Their lining epithelium was columnar and very regular; the apical protoplasm of the epithelial cells was finely granular, the basal protoplasm was more clear; the lumina often contained acidophilic granules or more homogeneous material (Fig. 6).

Cells of another type remain to be described, which, though present in small numbers in many places, completely dominated in others. They were relatively large cells with oval, more or less curved, bean-shaped or hook-shaped nuclei of medium density. The cells occurred as isolated elements or were arranged in small groups or cords. When

only two cells were lying together, the concave side of the nuclei faced each other. The isolated cells or the cell groups and cords were always surrounded by a fairly thick connective tissue membrane. In several places a large part of the cyst wall was almost exclusively occupied by closely packed, straight or curved cords of these cells, the cords being separated by thin septa of connective tissue (Figs. 7 and 8). The connective tissue which formed part of the cyst wall was edematous and contained fibrocytes, unstriated muscle cells, which were often arranged in bundles, and loose bundles of collagen fibers. In this connective tissue there were peculiar cell complexes which deserve a more detailed description. The complexes consisted of relatively small stellate cells with round to oval nuclei containing a small nucleolus. The cells were connected with one another by their processes. Very thin fibers, staining with aniline blue, were found in the protoplasm of these cells. The spaces between the cells appeared to be empty. Sometimes the cells lined cystic spaces, which contained little granular material. The whole structure resembled embryonal mesenchyme or the anlage of a lymph node. The stellate cells showed a tendency to be transformed into short spindle cells, which were lying close together, or into the larger cells, which resembled liver cells or interstitial cells (Figs. 9 and 10). Other cells remained smaller; their protoplasm was partly vacuolated, partly granular; a very few cells contained pigment reducing silver; their nuclei, however, resembled those of the larger cells; they occurred also in small groups and cords in the connective tissue. These cells resembled the interstitial cells which have been described in the hilum of the ovary (Fig. 11).

In one place a cystic space with a diameter of 1.5 mm. and containing a granular precipitate was found; it was partially lined by tissue composed of closely packed spindle cells (Fig. 12), small rounded cells, and interstitial cells; where the lining was thin it showed a superficial resemblance to an ovarian follicle.

In several blocks there were tubules and cysts which, though almost everywhere surrounded by, and in close contact with, the neoplastic tissue described above, were lined by an epithelium of a completely different type (Fig. 13). This epithelium varied from cuboidal to columnar and was in most places very regular; the nuclei were round, oval, or elongated and often contained conspicuous nucleoli. The free surface of many cells was covered by a striated border, under which there was a zone of homogenous protoplasm (Fig. 14). There were typical terminal bars. Here and there mitotic figures were found. Scattered between the epithelial cells were typical goblet cells with their thecae and stomata; they gave a positive reaction with mucicarmine.

Apart from the epithelial cells with and without a striated border and the goblet cells, a fourth epithelial cell type was found. These cells were also scattered between the other epithelial cells. The body of the cell sometimes made a bulge into the basal membrane; the cell body and nucleus were smaller than those of the other epithelial cells and the nucleoli were mostly very small or absent. The cells were more or less flask-shaped or triangular, the base of the cell resting on the basal membrane, the tip sometimes, but not always, reaching the surface. Sometimes the cells were more cylindrical.

In the basal protoplasm of these cells and only rarely higher than the nucleus, small yellowish granules were found. These granules stained deep orange-yellow with phosphotungstic acid hematoxylin and bright reddish yellow with safranine. With Masson's silver stain they stained brown-black (Figs. 15 and 16). Most of the tubules and cysts were completely surrounded by neoplastic tissue in which cells of the smaller type dominated. Others were lying in loose connective tissue. In this connective tissue there were larger and smaller groups of cells unlike any found in other parts of the tumor. The cells were smaller and often rounded or cuboidal, especially the cells lying in the outermost layer of the group. In the larger cell groups there were small lumina around which the cells were arranged in pseudorosettes (Fig. 17). Many of the cells, especially those bordering on the connective tissue, contained fine argentaffine granules (Fig. 18); with phosphotungstic acid hematoxylin they stained orange-yellow, as did the argentaffine cells found in the epithelium of the cysts and tubules.

Discussion

The diagnosis in this case cannot be considered difficult for almost all types of cells and tissues hitherto described in arrhenoblastomas were present. There were not only very typical tubules and interstitial cells, but also "sex cord-like" formations, atypical tubules and "sarcoma-like" areas (Fig. 12). The tubules depicted in Figure 4 resembled the tubules of the embryonal testicle, with the important difference that the conspicuous primitive sex-cells were absent. Formations like those in Figure 8 are depicted by Ewing [8] as testicular adenoma of the ovary. The interstitial cells were also quite typical; they contained silver-reducing pigment and resembled the interstitial cells of the embryonal testicle. Their origin from mesenchyme-like cells could be easily observed. Little importance can be attached to the fact that they did not contain crystalloids; in cases of tumor-like hyperplasia of the interstitial cells, crystalloids are often absent,[9, 10] and their occurrence in the interstitial cells of the normal testicle is not constant. The resemblance to liver cells has already been emphasized by Snell-

man [9] and Kaufmann.[11] The smaller vacuolated interstitial cells resembled the cells described by Berger [12] and Neumann [13] in close contact with nerves in the hilum of the ovary. The tubules with the regular columnar or cuboidal epithelium (Fig. 6) are identical with those found by Kleine [14] in several cases of arrhenoblastoma; they do not resemble structures found in the normal ovary or testicle.

The areas of small cells with darker or paler nuclei could be erroneously diagnosed as belonging to the diffuse variety of granulosa cell tumor. The presence of the numerous connective tissue fibers between the cells speaks against this diagnosis and Schiller [15] has pointed out that granulosa cell tumors and arrhenoblastomas cannot be differentiated from each other in the first immature phases. It must therefore be assumed that in my case both very typical and very immature neoplastic tissues were present; this is further proved by the finding of the mesenchyme-like areas. There were also many intermediate forms between the different cell types.

The tubules and small cysts, lined by the cuboidal or columnar epithelial cells with the striated border and by goblet cells, belong to an absolutely different type of tissue. The epithelium could not be distinguished from that of the intestine or other derivatives of the entoderm. That they were really of entodermal origin was proved by the presence of argentaffine cells. These cells occur only in the stomach, intestine, pancreatic duct, and gallbladder.[16, 17] As follows from the description and the photomicrographs, the cells in my case possessed all the characteristics of the argentaffine cells of the intestine, especially in regard to cellular form and to location and nature of the granules, which gave the typical staining reaction with Masson's silver method and with safranine. They were also very well brought out by phosphotungstic acid hematoxylin. The smaller and larger cell groups with the pseudorosettes and the argentaffine cells can only be diagnosed as a carcinoid; the location of the argentaffine cells in the different cell groups is typical for these tumors (see Masson's [7] Plate 6, Figs. 3 and 4). Whereas, for example, cartilage and unstriated muscle fibers can, at least in theory, develop everywhere in the mesenchyme and their presence be thus easily explained, this is not the case with entodermal epithelium and carcinoids. Black [18] and Mechler and Black,[19] who described a case of gynandroblastoma in which tubules with a more atypical epithelium showing a striated border and goblet cells were found, and which they did *not* compare with intestinal epithelium, adhered to the theory that the gynandroblastomas are teratomatous. Carcinoids have been found three times in ovarian tumors; these tumors were all dermoids—teratomatous tumors.[20] It is difficult to find

an explanation for the presence of the entodermal epithelium and the carcinoid in my case of arrhenoblastoma other than that of its teratomatous nature. When it is remembered that the pseudomucinous cystadenomas and the strumata ovarii are considered to be teratomas in which the pseudomucinous epithelium [21] or the thyroid tissue has dominated or blotted out the other teratomatous elements, there is nothing against assuming that in my case male-directed, hormone-producing elements have done the same, especially since Peyron [22] has proved that gonads may occur in a teratoma. Cases like that of Van Bouwdijk Bastiaanse,[23] in which an arrhenoblastoma was found in the wall of a pseudomucinous cyst, could be explained in the same manner. The origin of the teratoma itself remains, of course, open to discussion.

SUMMARY

In a very large, cystic arrhenoblastoma of the ovary entodermal elements, consisting of tubules and small cysts lined by epithelial cells with a striated border, goblet cells, and argentaffine cells, and a carcinoid were found. The entodermal elements were nearly everywhere surrounded by, and in close contact with, the cells belonging to the arrhenoblastoma. The case offers strong evidence for the theory that the arrhenoblastomas are of teratomatous origin.

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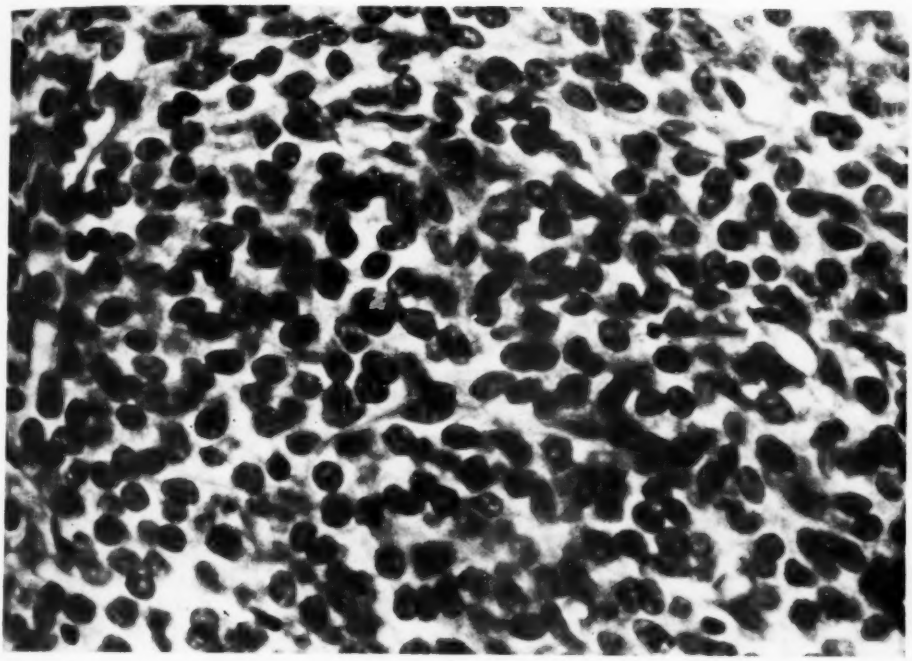
DESCRIPTION OF PLATES

The eighteen photomicrographic illustrations which follow were all prepared from the cystic arrhenoblastoma of the ovary which forms the subject of this report.

PLATE 204

FIG. 1. Cells of the small type which were the dominant element in many areas. × 715.

FIG. 2. The small cells in an alveolar arrangement. × 300.



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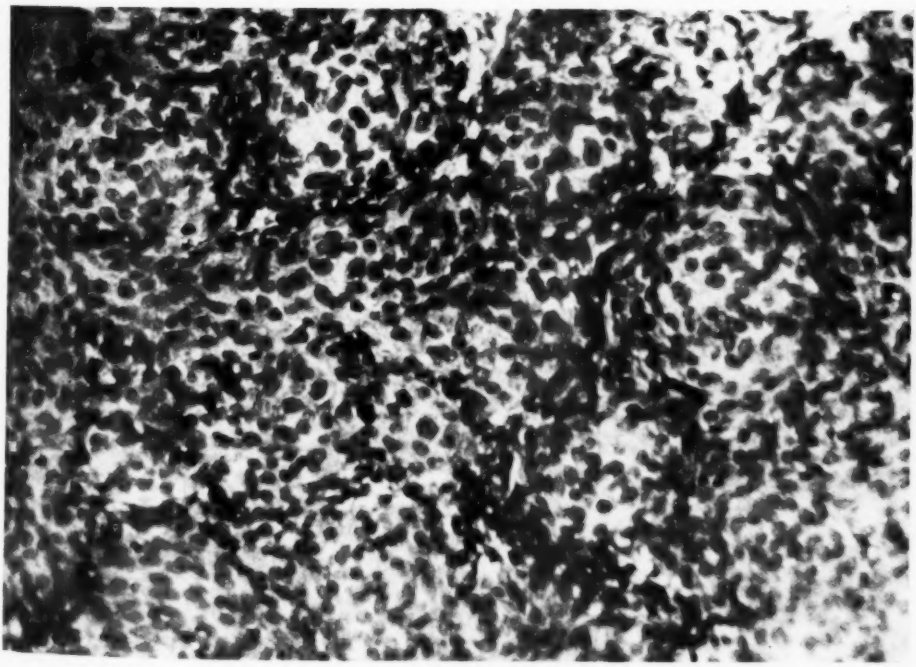

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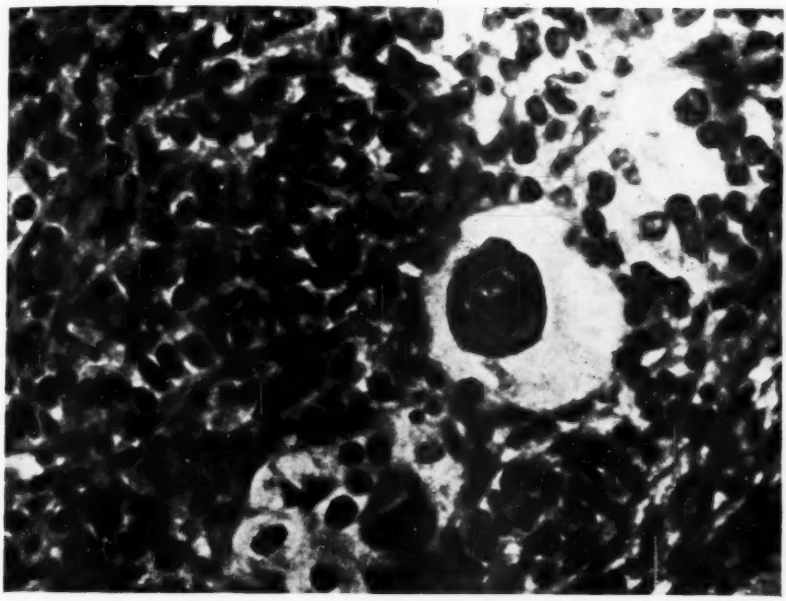
Arrhenoblastoma with Entodermal Epithelium

PLATE 205

FIG. 3. The giant cell form of the larger cellular type. × 715.

FIG. 4. Tubules resembling embryonal testis. × 715.



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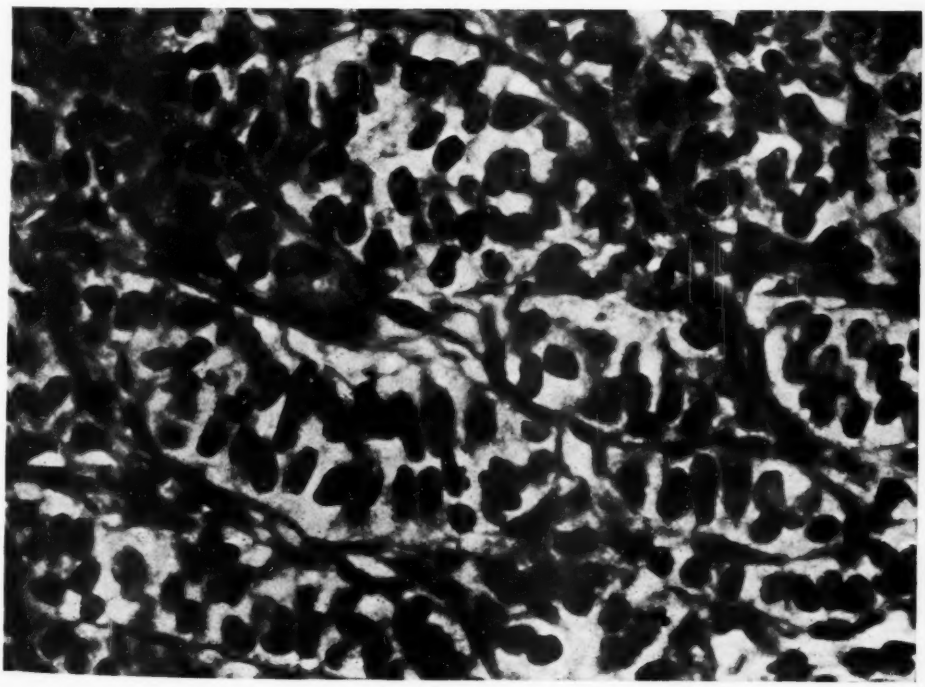

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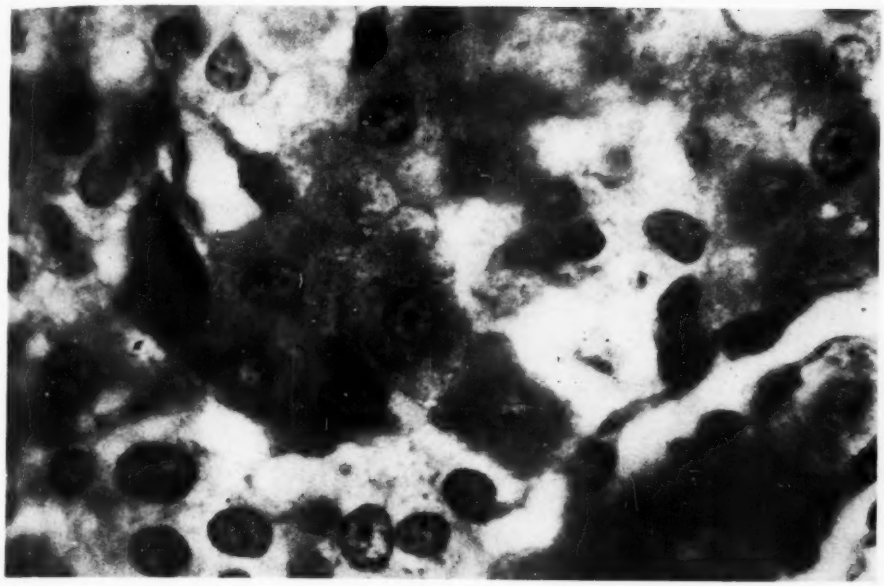
PLATE 206

FIG. 5. Interstitial cells containing isolated pigment granules. × 1275.

FIG. 6. Atypical tubules. × 610.



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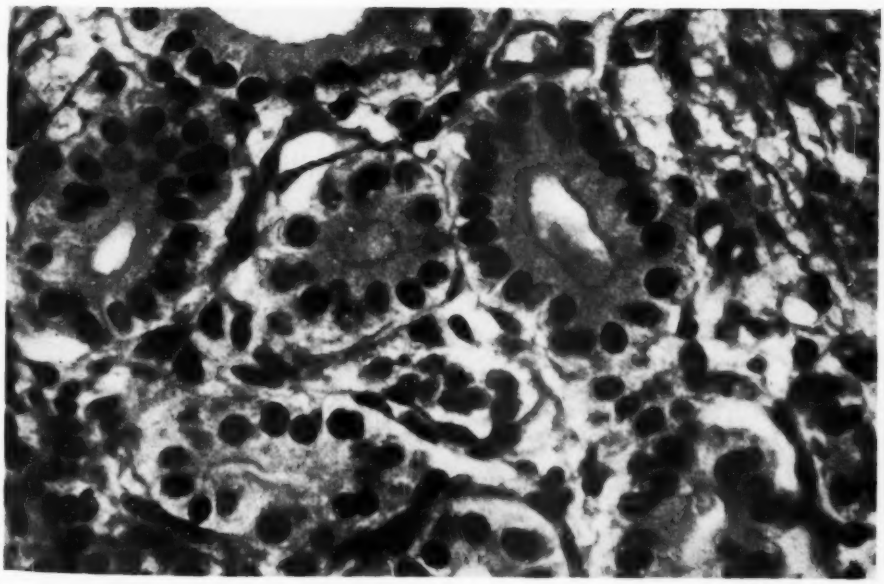

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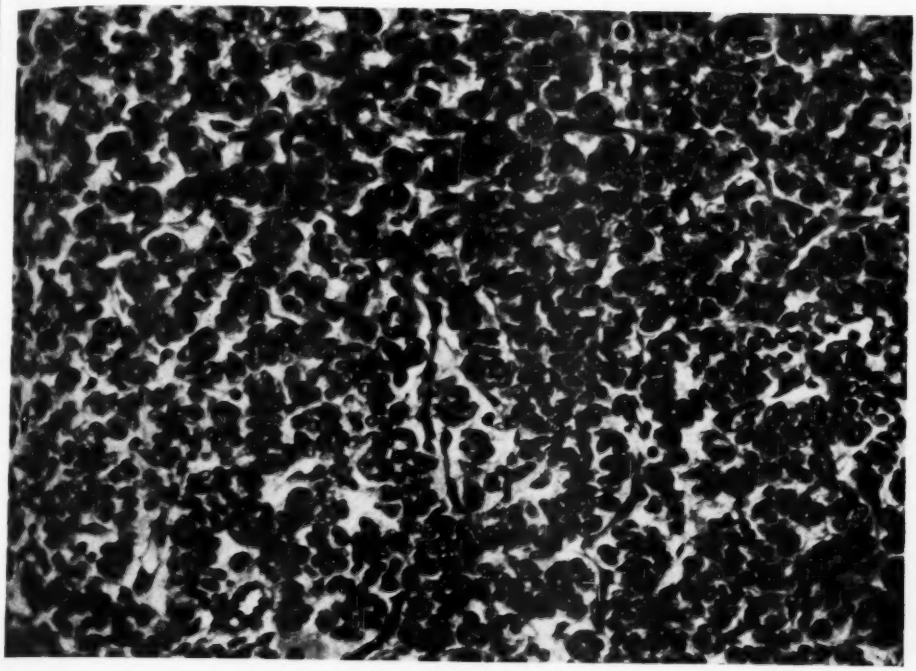
PLATE 207

FIG. 7. "Sex cord-like" formations. × 300.

FIG. 8. "Sex cord-like" formations. Acid alizarin blue-aniline blue stain. × 715.



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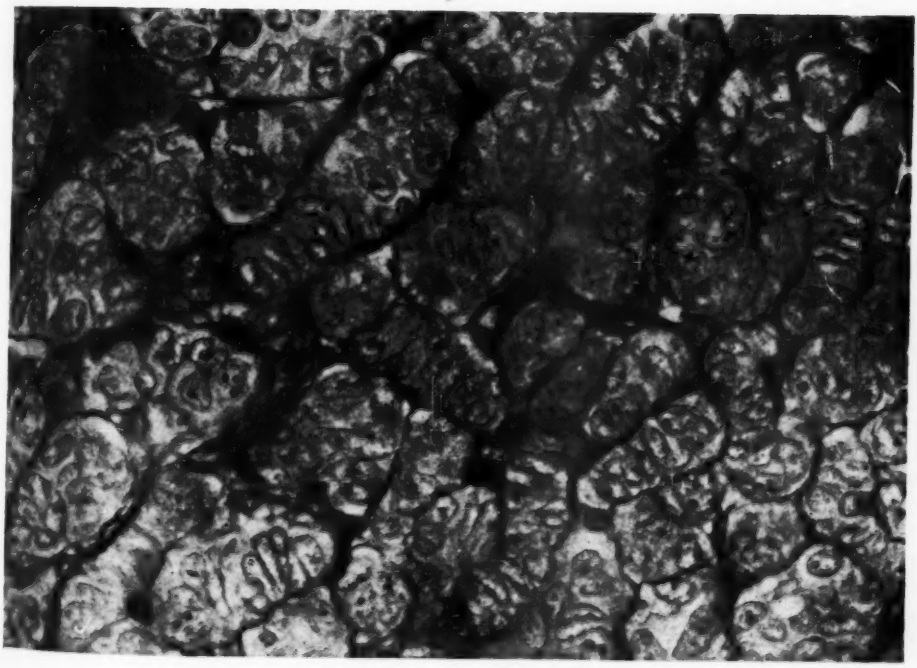

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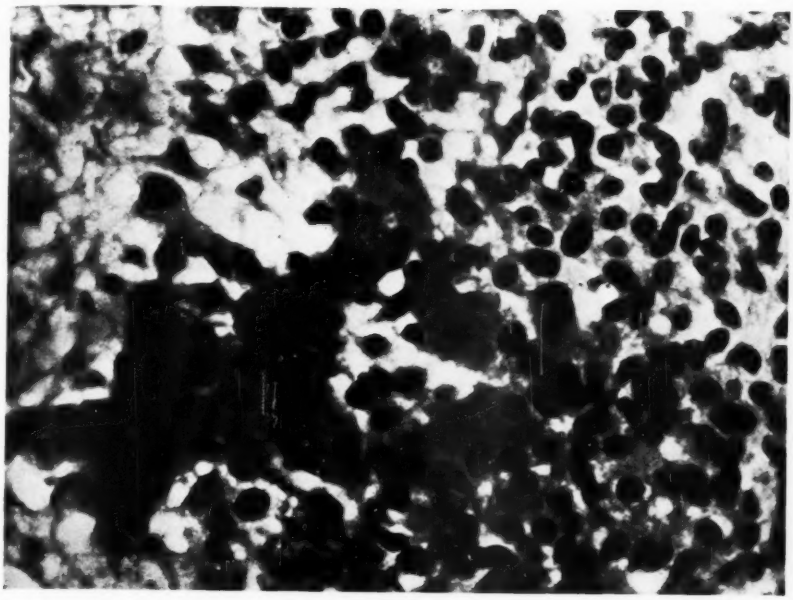
Arrhenoblastoma with Entodermal Epithelium

PLATE 208

FIG. 9. Interstitial cells, resembling liver cells, in a mesenchyme-like area. × 400.

FIG. 10. Interstitial cells differentiating from mesenchyme-like cells. × 1500.



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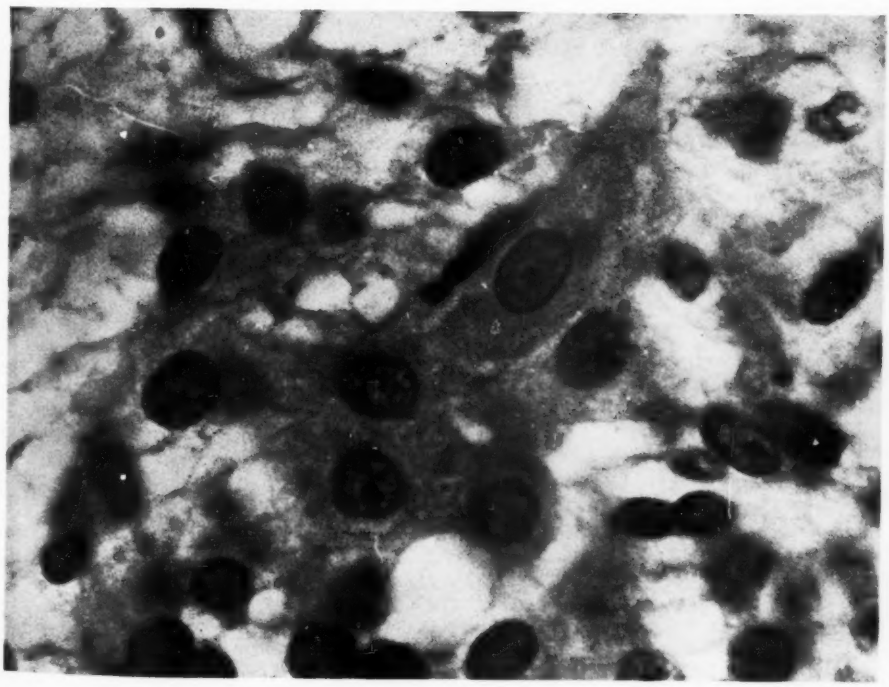

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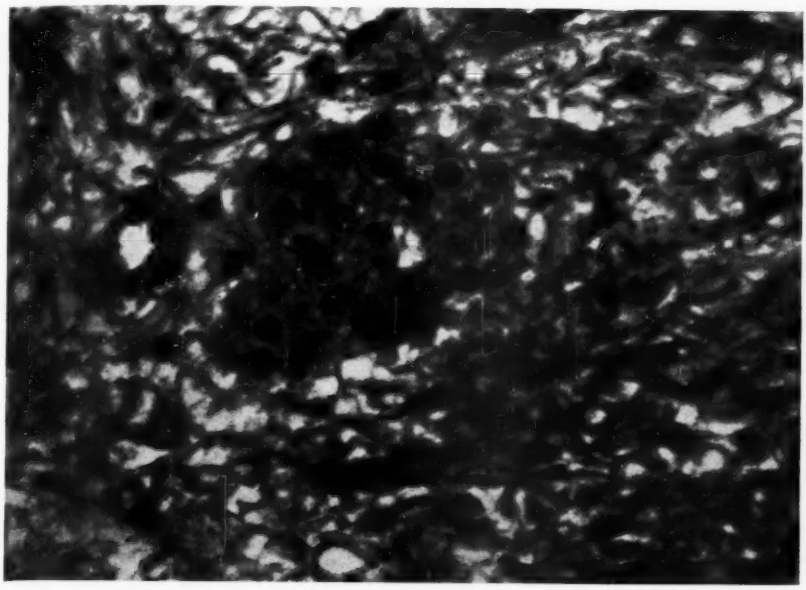
PLATE 209

FIG. 11. A group of small, vacuolated interstitial cells. × 400.

FIG. 12. A sarcoma-like area, with short spindle cells and one interstitial cell. × 715.



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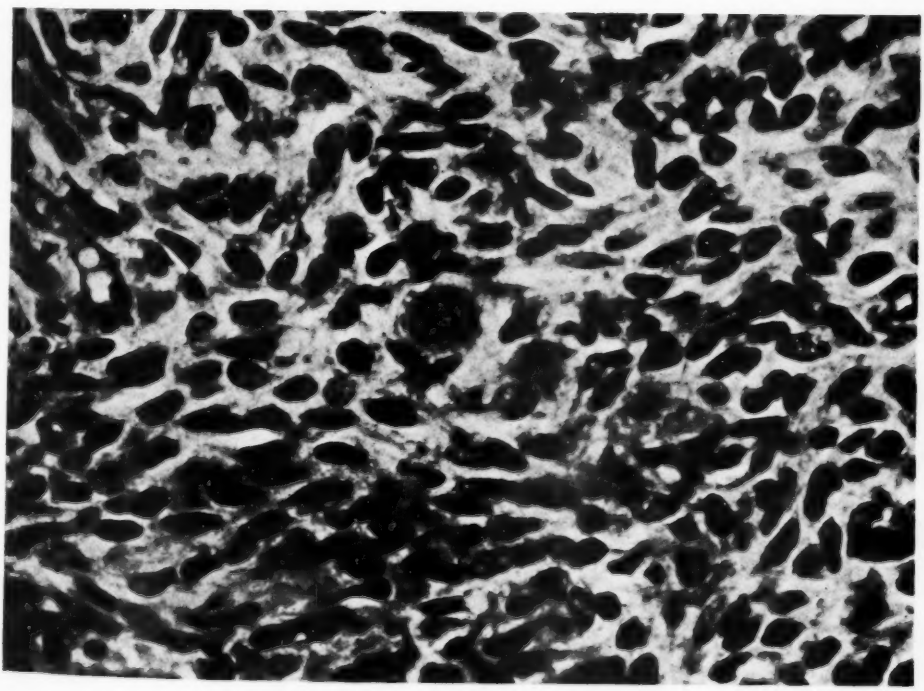

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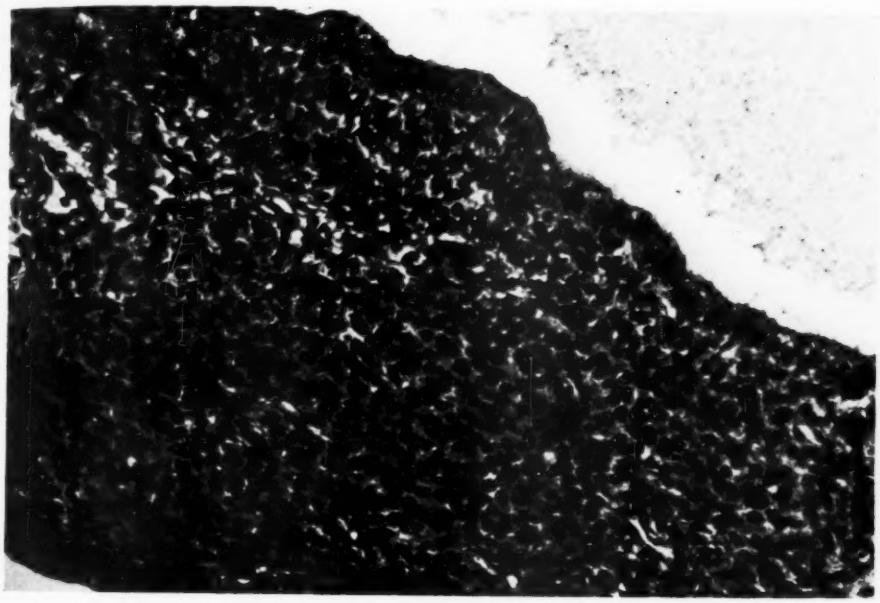
PLATE 210

FIG. 13. Wall of a cyst lined by entodermal epithelium and surrounded by neoplastic tissue of the small-celled type. × 260.

FIG. 14. High prismatic epithelium with a striated border. × 625.



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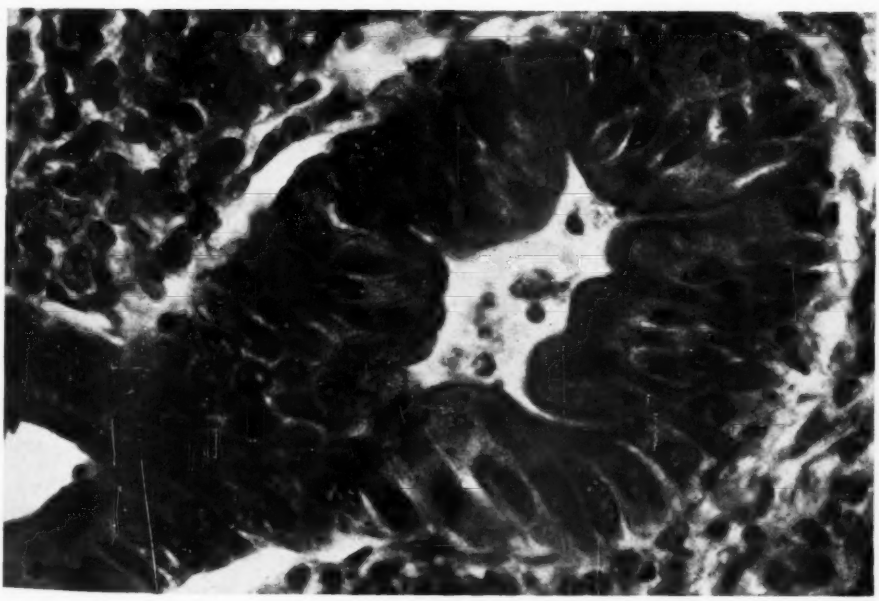

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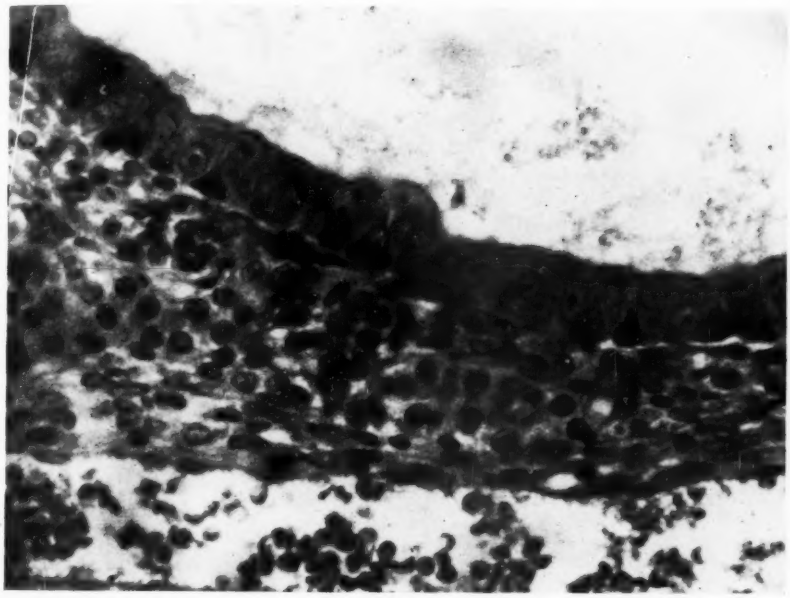
PLATE 211

FIG. 15. Entodermal epithelium containing argentaffine cells and surrounded by neoplastic tissue of the small-celled type. Masson's silver stain. × 500.

FIG. 16. Entodermal epithelium with typical argentaffine cells. Masson's silver stain. × 1250.



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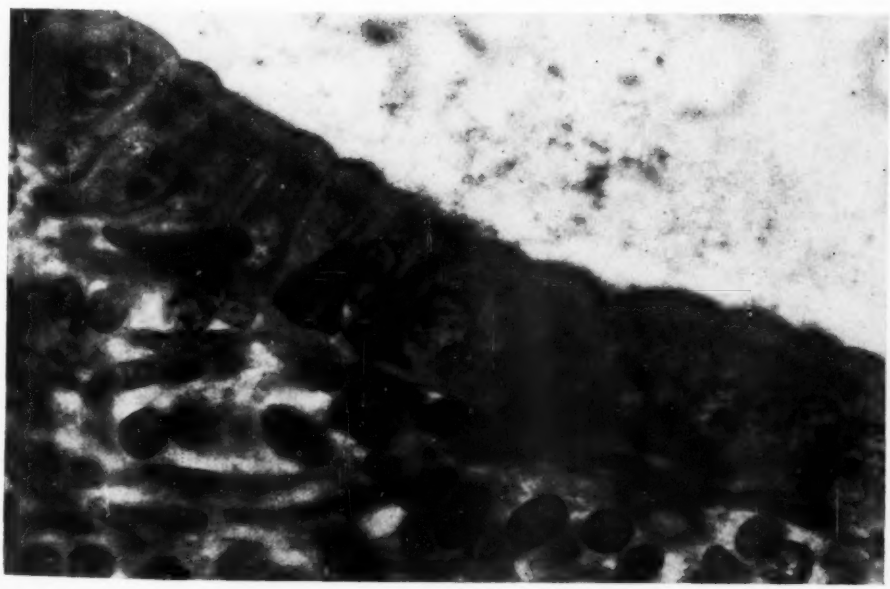

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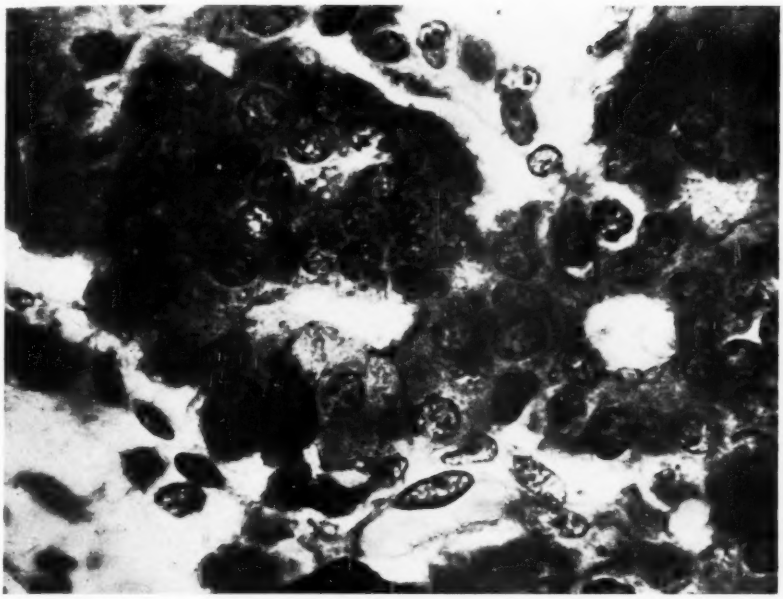
PLATE 212

FIG. 17. The carcinoid, showing pseudorosettes. Phosphotungstic acid-hematoxylin stain. × 900.

FIG. 18. The carcinoid, showing argentaffine cells. Masson's silver stain. × 900.



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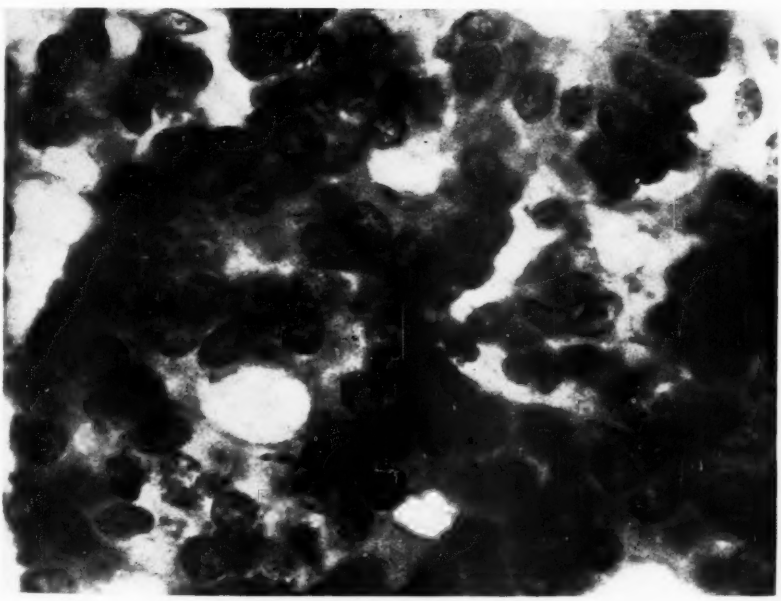

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Arrhenoblastoma with Entodermal Epithelium

TUBERCULOSIS OF THE MYOCARDIUM CAUSING COMPLETE HEART BLOCK *

T. Bhaskara Menon, M.D., and C. K. Prasada Rao, M.D.
(From the Departments of Pathology and Medicine, Andhra Medical College, Vizagapatam, India)

The rarity of tuberculosis of the myocardium is well established (Norris,[1] Raviart,[2] Horn and Saphir [3]). Gouley, Bellet, and McMillan [4] found approximately 200 cases in the literature. But tuberculosis of the myocardium involving the interventricular septum and causing heart block is so rare that the following case is of special interest.

Report of Case

Y. B., a well nourished woman of 35 years, was admitted to the Second Physician's ward of the King George Hospital on January 8, 1941. She complained of a peculiar sensation in the precordium, giddiness, and fainting fits of 7 months' duration. There was a previous history of intermittent fever for 2 months. During the fainting attacks there was no incontinence of sphincters nor a history of biting the tongue. Convulsive movements were also absent. On admission, the pulse was slow, bigeminal in type, with good volume and tension, the rate ranging from 30 to 40 per minute. There was no difference in the volume or rhythm on change of posture or exercise. Venous pulsation was noticeable at the root of the neck. The blood pressure varied from 170/110 to 154/90 mm. of Hg. The apex beat was not seen, but was felt in the fifth interspace in the midclavicular line and on percussion the left border was in the same position, other borders being normal. The heart sounds were slow. There was one ventricular extrasystole after every normal beat (coupled beats). A soft systolic murmur, heard at the mitral area, was conducted to the axilla. Other systems were largely normal. The urine showed more than a trace of albumin. The radiogram showed a prominent pulmonary conus with enlargement of the first part of the aorta. The patient died during an attack on April 8, 1941.

Gross Examination

Autopsy (Dr. C. S. Raju) showed a normal pericardium and a heart with increased fat under the epicardium. The weight of the heart was about 320 gm. There was a slight dilatation of the right auricle and ventricle and the myocardium appeared pale and had undergone fatty degeneration. The tricuspid ring was somewhat dilated. The tricuspid and pulmonary valves were normal. The left auricle and the mitral opening were of normal size. The aortic cusp of the mitral valve was slightly thickened and opaque and showed irregular calcified areas. Similar calcified opacities were found in the aortic cusps below their free margins. The coronary arteries showed moderate atheroma. A section of the interventricular septum revealed two whitish nodules in the muscle. One, vertically oval, measured 8 by 4 mm. and was near

* Received for publication, November 24, 1944. Because of difficulties in transportation, proof was not read by the authors.

the junction of the septum with the pars membranacea. The other was about 13 mm. below, in the thick muscular part of the septum. It was a horizontally oval mass measuring 4 by 2 mm. In serial sections these two nodules appeared irregularly continuous in the anterior part of the septum. The nodules were whitish yellow, firm, not encapsulated, but faded off into the surrounding pale myocardium. The tissue between the nodules was pale brown and rather friable, with small paler areas apparently in continuity with nodules. Further examination of the extent of the nodules could not be made without impairing the specimen.

The lungs showed some venous congestion, especially at the bases and posterior borders. There were pleural adhesions over the left lung but no nodules could be made out on section. The hilar lymph nodes showed only pigment deposit. The liver was normal in size and showed a smooth capsule, subcapsular mottling, and a faintly nutmeg appearance on section. The spleen was also normal in size and showed two old infarcts. The kidneys were slightly congested. Two small submucous leiomyomata were present in the uterus. The stomach and intestine showed nothing abnormal. There was slight congestion of the brain. Death was attributed to heart block followed by right heart failure. The nodule in the interventricular septum was regarded either as a gumma or a tuberculous nodule.

Microscopic Examination

The muscle fibers in the interventricular septum showed some increase in lipofuscin. A thin layer of hyalinized muscle fibers was found under the endocardium, but deeper down the muscle tissue was replaced by an irregular ramifying network of glistening collagen fibrils interspersed with lymphocytes and mononuclear cells with necrotic muscle fibers at the edges. There were numerous multinucleated giant cells, some of the typical Langhans' type and some of a more irregular foreign body type. Masson's stain showed that the fibers were of connective tissue type, staining with aniline blue. The giant cells in places were in clusters of two or three surrounded by ramifying fibers with a few lymphoid cells in between. In other areas the giant cells were arranged in systems of typical tuberculoid follicles. The possibility of a parasitic granuloma was considered, but no foreign material could be detected inside or outside the giant cells in serial sections. Small focal areas of lymphoid and mononuclear infiltration were found in the muscle bundles away from the granuloma. No endarteritis could be made out in the branches of the coronary vessels. Tubercles with

typical epithelioid cell-clusters and beginning caseation were more common in the periphery of the granuloma, while a more diffuse fibroblastic network was found in the center of the mass. Doubly refractile bodies could not be made out inside or outside the giant cells nor were fungal elements demonstrable. Staining by Jahnel's method showed no spirochetes. Examinations were made for acid-fast bacilli by various modifications of the Ziehl-Neelsen stain. They were found in scanty numbers lying free in the reticular mesh. Acid-fast bacilli were also independently demonstrated by Professor N. G. Pandalai of the Department of Bacteriology, so that the tuberculous nature of the granuloma was beyond question. Subsequently a careful histologic study of sections of various parts of the lungs showed a small tuberculous focus in the left lung lying under the pleura close to the apex.

Comment

Large caseous or conglomerate tuberculous lesions when found in the heart are generally in the auricular wall (Raviart,[2] Anders [5]) and are the result of extension from the hilar glands with pericardial involvement. Isolated myocardial foci deep in the muscle without pericardial involvement are generally regarded as due to hematogenous spread unless the endocardium is primarily involved. Even in valvular tuberculosis, involvement of the myocardium is not common. Baker [6] found only one instance in six of his reported cases. While continental authors regard subendocardial nodules as not very uncommon in general miliary tuberculosis, deep myocardial tubercles are rarely found. This rarity of deep myocardial tuberculosis may be due to the difficulty of development of a focus in an active contractile tissue.

A feature of interest in this case is the probable hematogenous origin since no pericardial or endocardial focus could be demonstrated after careful examination. The only other focus in the body which could be regarded as histologically active was a subpleural nodule. There was nothing to suggest that this was secondary to rupture of the myocardial lesion. The presence of this subpleural focus rules out the possibility of a primary myocardial tuberculosis such as was reported by Berger and Miller,[7] by Gunewardene and Gunewardene,[8] and others. The lack of involvement of the mediastinal glands also suggests an accidental myocardial localization from the blood stream. Wilbur [9] has reported four cases of myocardial tuberculosis, in two of which the infection was apparently hematogenous. The tubercles appeared in small scattered foci and in one case healing had given rise to interstitial myocarditis. The location of the nodules in the present case in the

interventricular septum, giving rise to complete heart block, is a feature of great interest, while the absence of tubercles in the rest of the myocardium is also a noteworthy feature.

The patient was admitted under the care of one of us (C.K.P.R.) but subsequently, for a short while, came under the care of Dr. R. Viswanathan to whom our thanks are due.

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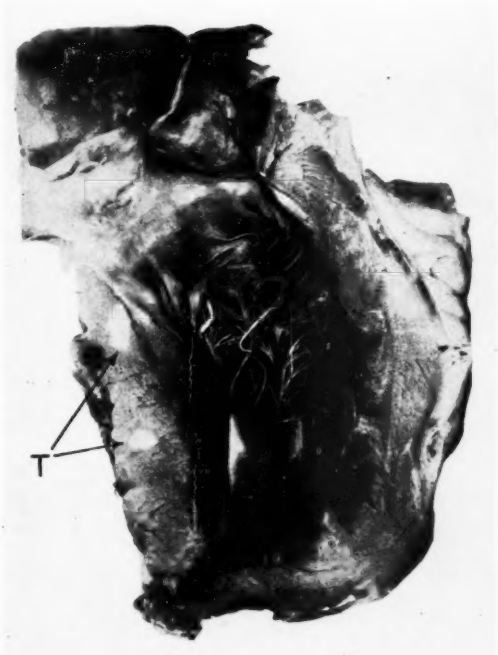
DESCRIPTION OF PLATE

PLATE 213

FIG. 1. Two tuberculous nodules in the interventricular septum. The right ventricle has been removed.

FIG. 2. Tuberculoid follicles at the periphery of the granuloma in the heart muscle. Hematoxylin and eosin stain. × 80.

FIG. 3. Subpleural nodule with tubercles. Hematoxylin and eosin stain. × 80.



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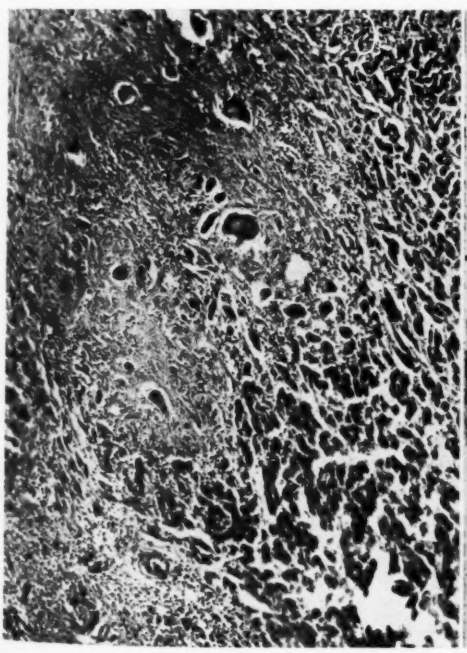

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VOLUME XXI, No. 6 WHOLE NO. 127

NOVEMBER, 1945

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